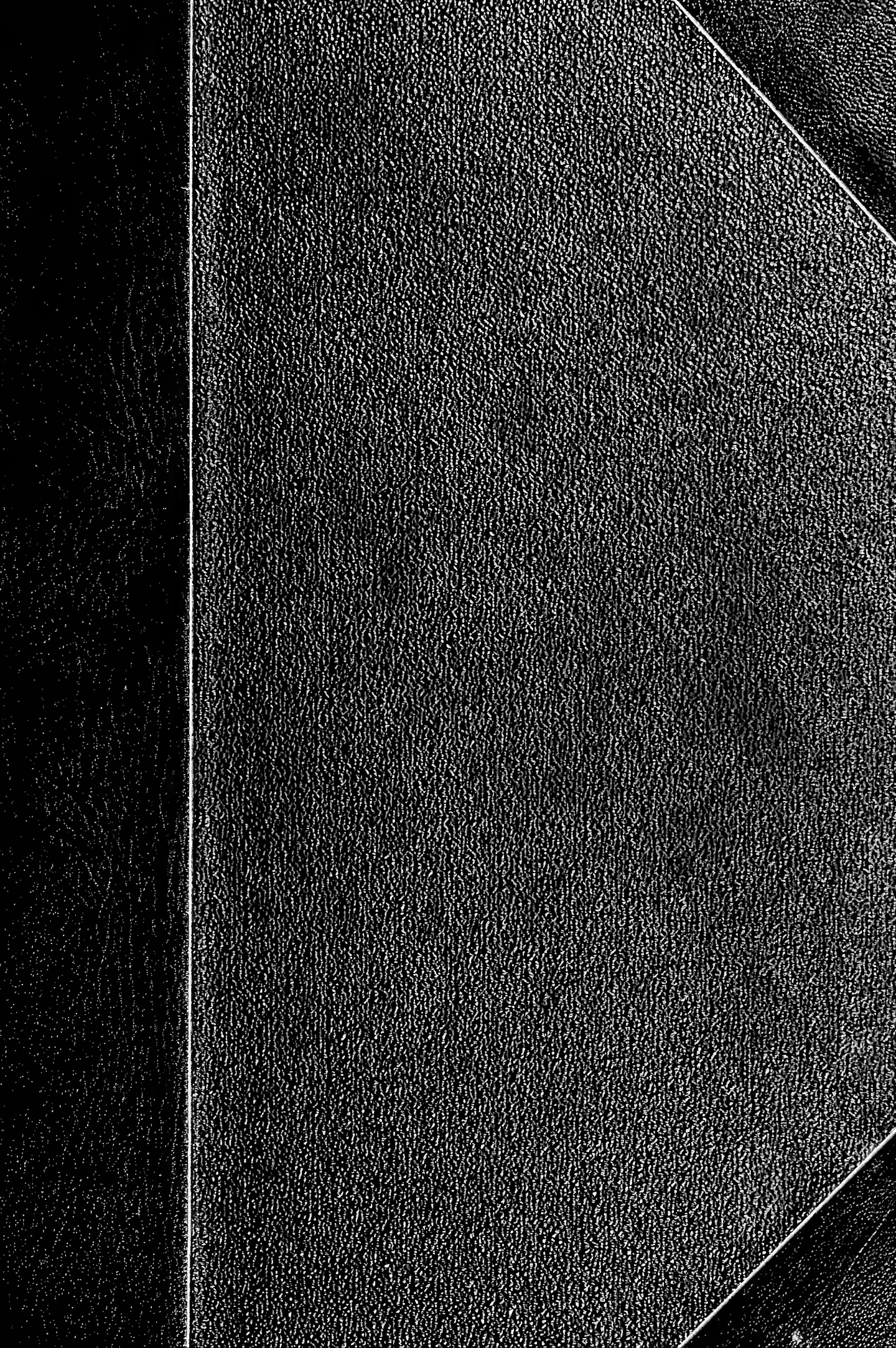

PARASITOLOGY

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A SUPPLEMENT TO THE

JOURNAL OF HYGIENE

EDITED BY

GEORGE H. F. NUTTALL, F.R.S.

Quick Professor of Biology in the University of Cambridge

AND

A. E. SHIPLEY, F.R.S.

Reader in Zoology in the University of Cambridge

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NOTES ON TICKS. I.

(1) *IXODES CALEDONICUS*, DESCRIPTION OF MALE, TOGETHER WITH CONSIDERATIONS REGARDING THE STRUCTURE OF THE FOOT IN MALE *IXODES*.

(2) TYPES OF PARASITISM IN TICKS, ILLUSTRATED BY A DIAGRAM, TOGETHER WITH SOME REMARKS UPON LONGEVITY IN TICKS.

(3) REGARDING THE LOSS OF LIFE IN TICKS OCCURRING ON WANDERING HOSTS.

BY GEORGE H. F. NUTTALL, F.R.S.

(*From the Quick Laboratory, University of Cambridge.*)

(2 Figures.)

I. *Ixodes caledonicus etc.*

IN a paper published last year[1], I described the female, nymph and larva of *Ixodes caledonicus*. Having regard to the views expressed in my recent paper on the adaptation of ticks to the habits of their hosts[2], I wrote to Mr William Evans, in Edinburgh, asking him, if possible, to kindly search the dove-cot at Dunipace, Stirlingshire, for the missing male which he would probably find in the habitat of the host, the domestic pigeon. On 25 May, 1911, Mr Evans discovered two ticks, a male and female in copula, in the dove-cot, and he sent the specimens to me for identification. The female accords with my published description of *caledonicus*. The male is here described for the first time,

[1] *Parasitology*, III. 408–411, Figs. 1–3; reprinted in *Ticks*, Part II, 198–200, Figs. 191–193.

[2] *Parasitology*, IV. 46–67, Figs. 1–26; reprinted in *Ticks*, Part II, 324–345, Figs. 289–307.

and it is interesting to find that it agrees in structure and habits with what was to be expected according to the theory advanced by me in the second paper I have referred to.

Mr Evans writes that he found the young pigeons in the nests at Dunipace had many of the ticks (nymphs and females) upon them, chiefly attached about the head and anus. On removing some three or four nests and the young birds they contained, he collected as much as he could of the *débris* and filth from beneath the nests, placed it in a paper bag and examined it in the open by spreading it out on a sheet of white paper exposed to the sun's rays. Apart from fleas and other creatures, he succeeded in collecting about 100 ticks from amongst the rubbish, *i.e.*, 3 ♂ s, 4 gorged ♀ s, many unfed ♀ s and ○ s, and a few larvae of *I. caledonicus*. In only one case, as noted above, were the sexes found in copula.

It is interesting to record, moreover, that Mr Evans has, more recently, sent me for determination 1 ♀ and 6 ○ s (unfed and replete) of *I. caledonicus* which he found on a dead *starling*, in the Isle of May, Fife, Scotland, in May, 1911.

In common with *hexagonus, canisuga, putus* and *vespertilionis*, the male of *caledonicus* is characterised by its large size, compared to the unfed female, by the relatively small capitulum, by the small size of the pads on the feet, whilst the unarmed hypostome recalls the condition observed in *putus* and *vespertilionis*. The tick has other points of affinity with several of these species: the capitulum resembles that of *vespertilionis* but for the palps, which are not clavate; it is the only species of *Ixodes* other than *vespertilionis*, in which the median ventral plate is separated from the anal plate by the interposition of the anterior portion of the adanal plates; the character of the palps (excavate) and the short legs of *caledonicus* ♂ give it an intermediate position between *vespertilionis* ♂ and the males of other species of *Ixodes*, thus affording further evidence in support of the view expressed by Nuttall and Warburton (*Ticks*, Part II, p. 134, 1911) that the genus *Eschatocephalus*, created solely for *vespertilionis*, should be suppressed in favour of *Ixodes*. The hairs along the posterior ventral border in *caledonicus* and other characters suggest a resemblance to *putus*; the two deep dorsal depressions, from which arise grooves running to the posterior border, recall the appearance seen in *canisuga*.

In this connection I would note the following as bearing on the structure of the foot in *Ixodes* males. On examining the males of species in which *both sexes are known to occur upon the host*, I find that

the foot is provided with a *large pad*, equal or nearly equal in length to the claws. I have examined males of the following 11 species belonging to this category: *ricinus, rasus, schillingsi, pilosus, boliviensis, cavipalpus, rubicundus, minor, loricatus, angustus* and *holocyclus*, leaving only one species in which the foot structure remains undetermined. In all the species I have named the pad is large, whereas in *caledonicus, canisuga, hexagonus, putus* and *vespertilionis*, the pad is small, these being species wherein the sexes are not recorded as occurring together upon the host. It is clear that the large pads on the feet are correlated with the parasitic habits of the males in the preceding group, their presence ensuring a firmer foothold for the tick when upon the host[1].

It is evident that the structure of the foot in ticks requires further study in relation to the habits of parasitism characterizing the various stages. In Ixodidae, other than *Ixodes*, the pads appear to be of similar size in both sexes, and this is to be expected for both sexes in these species occur upon the host.

Ixodes caledonicus Nuttall, 1910.

Male (Fig. 1). Body oval, dark brown, $3{\cdot}2 \times 2{\cdot}1$ mm. *Scutum* hairless, glossy, with rounded protruding scapulae, slightly constricted antero-laterally close behind where the marginal fold appears, the latter broad behind; cervical grooves distinct, straight, slightly divergent behind, ending near the anterior third of the scutum and followed, after a short interval, by two large depressions whence proceed coarsely punctate grooves diverging slightly and extending to near the posterior border; the areas between the grooves protuberant, with only very minute, almost invisible punctations. *Venter*: genital orifice between coxae II; pregenital plate small, broader than long, indented in front; median plate small, widely separated from the anal plate by the adanal plates intervening (as in *I. vespertilionis*), with two lateral longitudinal grooves; anal plate rounded in front, with sides curved and converging slightly posteriorly; adanal plates large, long, broad in front, fusing in the median line *in front* of the anus, with sides nearly parallel; all the plates very glossy, with few minute punctations; *short, coarse hairs*

[1] In the larval *Argas persicus*, as has been noted elsewhere, the pads on the feet are large. The tick in this stage clings to the host for some days, or longer. In the nymphs and adults (and this appears to apply to all Argasidae) the pads are small, these stages being characterized by their habit of feeding rapidly. It appears to follow, also in this instance, that the presence of the large pad in the larva is correlated with its parasitic habits.

occur only near the posterior border of the body and extend along the sides to near the spiracles. Spiracles medium, rounded. *Capitulum* 0·3 mm. long[1], very small compared to the body; base with rounded sides, narrowing behind; dorsal ridge narrow, straight, not attaining the sides and continuous from the external angles with slight lateral ridges which converge anteriorly; base convex ventrally, no ventral ridge; no auriculae. Palps short, slightly excavate, converging and blunt distally (asymmetric in the type), considerably longer than the hypostome, with article 1 distinct dorsally, articles 2 and 3 fused, article 4 appearing as a relatively large chitinized plaque. Hypostome short, glossy, unarmed,

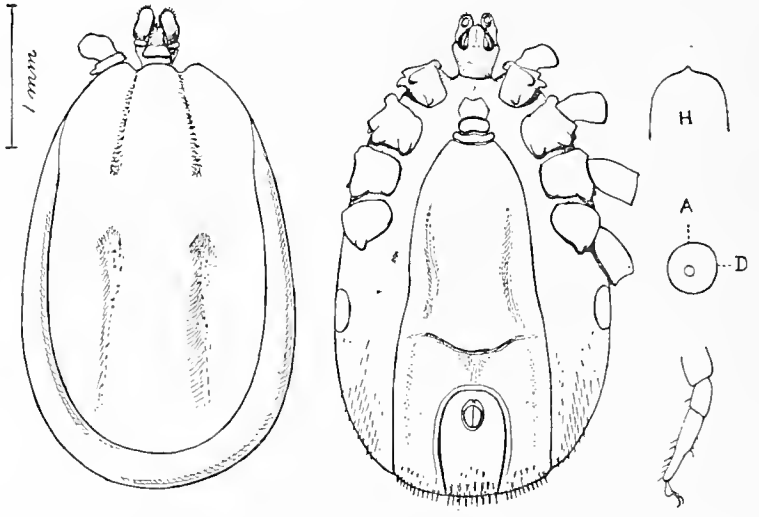

Fig. 1. *Ixodes caledonicus* Nuttall, 1910; ♂ dorsum and venter; (*H*) tip of hypostome, highly magnified; spiracle and tarsus 4.

with median longitudinal ridge, bluntly rounded in front with a small median prominence. *Legs*: all the coxae bear *two* stout postero-external spurs, the one *dorsal*[2] to the other and decreasing in size backward; coxae I–III with short postero-internal trenchant spur decreasing in

[1] Measured from the dorsal ridge to the tip of the hypostome.

[2] The presence of the prominent *dorsal* postero-external coxal spur in the male led me to re-examine the ♀, o and L, to see if I had overlooked this peculiar structure in them. I find that the ♀ shows these spurs but that they are less developed than in the ♂. In the *female* they are present on coxae I–III, very slight on coxa III, and absent on coxa IV. In the *nymph* they are present on coxa I, slight on coxa II, absent on coxae III–IV. In the *larva* they are absent. Although these spurs are easily overlooked it is well to note them in connection with the description I have already published of the ♀, o and L of *caledonicus*.

size backward, absent on coxa IV which is rounded; coxa I visible dorsally owing to its protruding anterior angle; trochanters *unarmed*[1]; movable articles slender; tarsus 4 tapering obliquely, claws much longer than the pad.

II. Types of parasitism in ticks illustrated.

Having frequently had occasion to explain to students the different types of parasitism observed in ticks, it occurred to me recently that these types could, with advantage, be illustrated graphically for the purpose of instruction.

The life-cycles of the ticks are represented in the accompanying diagram, Fig. 2, in the form of a dial, the various stages being named at the periphery; lines running to the centre enclose corresponding areas

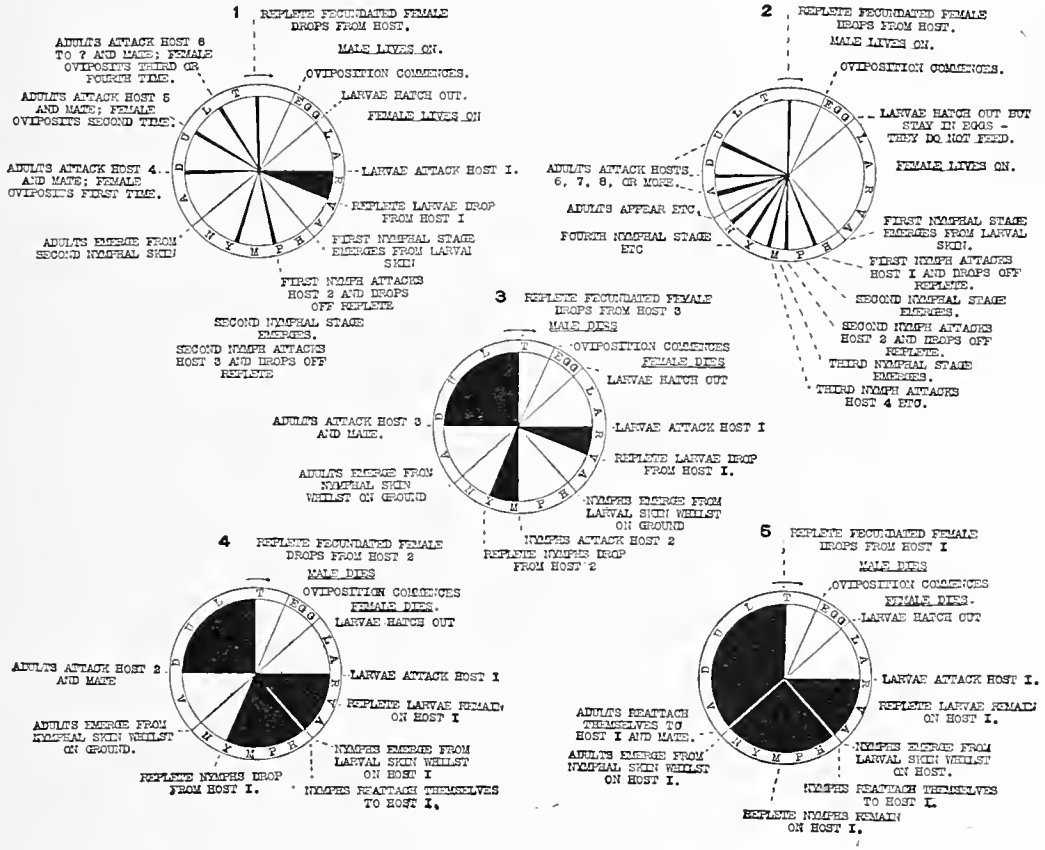

Fig. 2. Diagrams representing graphically the different types of parasitism observed in ticks (refer to text).

[1] Differing in this respect from the female, nymph and larva.

indicating the parts of the cycle representing each successive stage in the tick's development, commencing with the ovipositing female, after which follow the egg, larval, one or more nymphal and, finally, the adult stages (more especially the females). The blackened parts of the dial indicate the part of the life-cycle which is spent in sucking the blood of the host. The numbers alongside the five dials correspond to the following types of parasitism :—

Type 1, represented by *Argas persicus*, also probably by *A. reflexus* and *A. vespertilionis* and some other species of *Argas* and *Ornithodorus*. It will be seen that only the larval stage remains attached to the host for some time (5 to 7 days, or more) whilst sucking blood. The later stages are rapid and repeated feeders. There are at least two nymphal stages; the adults attack a succession of hosts and feed repeatedly, laying eggs in batches after each feed. There are many breaks in the chain of parasitism.

Type 2, represented by *Ornithodorus moubata* and *O. savignyi*. The larva does not feed. The first stage nymph and the succeeding nymphal stages and adults are all rapid feeders and attack a number of hosts in succession. But for the inactive larval stage these ticks behave similarly to the ticks under Type 1, there being many breaks in the chain of parasitism.

N.B. An aberrant type of parasitism is that of *O. megnini*, in that the larval and nymphal stages (probably two or more of the latter) feed, whereas the adults do not. This type is not illustrated in the diagram, it has been fully described elsewhere[1].

Type 3. This appears to represent the commonest form of parasitism in Ixodidae. It has been observed, for instance, in the following species, which have been raised experimentally: *Ixodes hexagonus, I. ricinus, I. canisuga, Haemaphysalis leachi, H. punctata, Dermacentor reticulatus, D. occidentalis, Rhipicephalus appendiculatus, R. sanguineus, R. simus* and *Amblyomma hebraeum*. Here the tick attacks and feeds upon three successive hosts upon which at each stage (larva, nymph and adult) it remains attached for some days, or more, until it becomes replete and abandons the host. There are two breaks in the chain of parasitism between the larval and adult stages.

Type 4, represented by *Rhipicephalus evertsi* and *Hyalomma aegyptium*. Here the larva and nymph are parasitic upon one host and the adult attacks a second host. Whilst upon the first host there is a short interval (represented in the diagram by a fine white line traversing

[1] *Parasitology*, III. 52–56, reprinted in *Ticks*, Part II, 330–334.

the black) when the tick ceases feeding, *i.e.* during metamorphosis from larva to nymph; disregarding this, there is but one break in the chain of parasitism between the larval and adult stages.

Type 5, represented only by species belonging to the genus *Boophilus*. Here the tick runs through its whole life-cycle, from larva to adult, upon one host. The feeding upon this host is twice interrupted (represented in the diagram by two fine white lines traversing the black) for a brief period during the metamorphosis from the larval to the nymphal and from the nymphal to the adult stages. There is no break in the chain of parasitism between the larval and adult stages.

In types 1 and 2 we have ticks which feed in succession on an indefinite number of hosts. In types 3 and 4 the ticks require to find three or two successive hosts respectively, whereas in type 5 but one host is required.

The *longevity* of a species differs according to the type of parasitism it possesses. In species which attack numerous hosts in succession (*Argasidae*: types 1 and 2) the longevity is greatest and it is least in species (*Boophilus*: type 5) requiring but one host. Ticks belonging to types 3 and 4 presumably occupy an intermediate position in this respect. It is obvious that in a species requiring a succession of hosts the tick will fast repeatedly for variable intervals of time and that it must be greatly to its advantage to be able to withstand prolonged starvation. Longevity may be measured in two ways: (*a*) upon the unfed tick at different stages, noting how long it survives as a larva, nymph and adult respectively, reckoning from the time of its emergence; (*b*) upon the time it takes for the tick to complete the cycle of development, starting at any stage and ending with the reappearance of that stage. We have, unfortunately, too few data at present at our disposal to permit of any generalization regarding the longevity of ticks measured by either of these methods. We know of Argasidae which may survive 2–6 years as unfed adults, this longevity being unequalled in the Ixodidae, unfed adults of which usually survive but 6 or 8 months. Argasidae may run through their life-cycle within a year or two under the most favourable conditions, but in nature this period must frequently be very greatly prolonged. On the other hand, in Ixodidae we find considerable differences in longevity, as measured by the life-cycle, even under what appear to be the most favourable conditions: thus the shortest period reckoned for the cycle of *I. ricinus* is 178 days, of *R. appendiculatus* it is 77 days, both species belonging to type 3 in the diagram. *Boophilus* may run through its cycle in from 21 to 23 days.

III. Regarding the loss of life in ticks occurring on wandering hosts.

In acknowleding the receipt of my paper, entitled, "On the Adaptation of Ticks to the Habits of their Hosts," Mr E. G. Wheler writes from Claverdon Leys, Warwick, April, 1911, that he does not consider the loss of life in ticks which occur on wandering hosts is as great as might be supposed. "If the habits of cattle, sheep, etc. are watched, they will be found to be guided by very great regularity. They feed in the same places at the same time of day, lie down in the same part of a field or moor, and even take a regular round of their ground day by day. All these movements are governed by the weather, and are subject to variation accordingly; but taking the year round, the chances of ticks finding a host are much greater than if the habits of the hosts were purely haphazard in character.

As a typical example, I often used to stay at a vicarage near Chillingham, and in sight of one part of the Park. The wild cattle were, in fine weather, always visible from the house at the same time in the afternoon, never in the morning, and the portion of the Park in question was only a few acres in extent.

Again, in Alnwick Park, where I collected most of my ticks (*I. ricinus*) for experimental purposes, I rarely found any except in patches of long rushes, where the deer lay habitually in the summer months because the stags could brush the flies off their horns, then in velvet, and except for a few weeks in the spring and again in the autumn, very few were to be found, showing plainly that they had soon found a fresh host. Of course, a certain number must fall off in other places, but I fancy the proportion is smaller than might be expected, taking into consideration the amount of unsuccessful searching I have done in unlikely places.....At Alnwick Park a portion of the Deer Park was fenced off, and after two years it was practically clear of ticks. I think after a *long* search I found two where they were abundant before the deer were excluded. It has a possible bearing on the time required for extermination of *I. ricinus*."

THE RELAPSING FEVER OF TROPICAL AFRICA. A REVIEW.

By EDWARD HINDLE, Ph.D., A.R.C.S., F.L.S.
Beit Memorial Research Fellow.

(*From the Quick Laboratory, Cambridge.*)

(With two Maps and one Chart.)

Introduction. In the following pages an attempt has been made to give a short summary of our knowledge of the Relapsing Fever of Tropical Africa, and also to include the results of my own experiments and observations on this disease. The literature on the subject is already somewhat considerable and widely scattered, therefore no apology is needed for bringing it together.

The term "Tick-Fever" has been employed to denote so many different diseases (*e.g.* piroplasmosis, spirochaetosis, tick-bite fever, etc.) in both man and animals, that it is hoped the name will be dropped from the literature, as it is too ambiguous to be of any use. The term "Relapsing Fever of Tropical Africa" has been employed throughout the present paper to denote the disease caused by *Spirochaeta duttoni*, and this name will serve to distinguish it from the other relapsing fevers of Africa, such as those of Abyssinia, Algeria, etc.

History. The fact that the bite of *Ornithodorus moubata* is sometimes followed by more or less serious symptoms, has been known for more than 50 years. Thus Livingstone (1857) mentions the disease, and describes the effects of the tick's bite in the following words:— "When sleeping in the house of the Commandant (of Ambaca, Angola) an insect, well known in the Southern country by the name *Tampan*, bit my foot. It is a kind of tick, and chooses by preference the parts

between the fingers or toes for inflicting its bite. It is seen from the size of a pinshead to that of a pea, and is common in all the native huts in this country. It sucks the blood until quite full, and is then of a dark blue colour, and its skin so tough and yielding, that it is impossible to burst it by any amount of squeezing with the fingers. I had felt the effects of its bite in former years, and eschewed all native huts ever after, but as I was here again assailed in a European house, I shall detail the effects of the bite. These are, a tingling sensation of mingled pain and itching, which commences ascending the limb until the poison imbibed reaches the abdomen, where it causes violent vomiting and purging. Where these effects do not follow, as we found afterwards at Tete, fever sets in; and I was assured by intelligent Portuguese there, that death has sometimes been the result of this fever. The anxiety my friends at Tete manifested to keep my men out of the reach of the *tampans* of the village, made it evident that they had seen cause to dread this insignificant insect. The only inconvenience I afterwards suffered from this bite, was the continuance of the tingling sensation in the point bitten, for about a week" (pp. 382–383).

He also writes:—"We had heard frightful accounts of this insect[1] while among the Banyai, and Major Sicard assured me that to strangers its bite is more especially dangerous, as it sometimes causes fatal fever" (pp. 628–629).

As far as I am aware this is the first record of the fact that an attack of fever may follow the bite of the "human tick."

Some years later Sir John Kirk again mentioned this disease which he found in the Zambesi valley as far up as Sescheke, above the Victoria Falls, and in North-Western Rhodesia. He writes: "The symptoms appear soon after the bite, and are sharp fever, vomiting and often delirium; in about two days these pass off, but there is no marked profuse perspiration as in malarial fever. After recovery, the patient has complete immunity from further attacks, however he may be bitten, but it is doubtful whether this immunity lasts for any length of time in case of removal."

Dr Hinde (1897) in an expedition to the Congo Free State in 1892–1894, saw sick persons who attributed their illness to the bites of the human tick; but, although some of his own men died from the same cause, he believed that they had died through the force of their superstitions, and not from the effects of the tick. As a result any natives who complained of suffering from tick-bites were treated as malingerers!

[1] *Tampans*, called '*Carapatos*' at Tete.

From time to time other writers recorded the existence of "tick-fever" in various parts of Africa, and in 1903, Christy suggested that the disease was caused by a filaria carried by the tick.

Nabarro in 1903 noticed the presence of spirochaetes in the blood of a boy from Uganda suffering from this fever, but as his results were not published until 1905 (Nabarro and Greig, 1905), he lost priority for his discovery.

Cook (1904) was the first to definitely record the presence of a "spirillum" closely resembling *S. recurrentis* in the blood of patients suffering from the disease. Although this author gives a good description of the symptoms of the fever, he failed to recognise its identity with "Tick-Fever," but supposed it to be the same as European Relapsing Fever. The same year P. H. Ross and Milne (1904) found "spirilla" in the blood of eight natives of Uganda suffering from "Tick-Fever," and recognised them as being the cause of the disease. Independently of these observers, Dutton and Todd (1905) at the same time had been carrying on observations on this fever in the Congo Free State and had discovered that it was caused by a spirochaete occurring in the blood, and that the infection was conveyed by the bite of the human tick, *O. moubata*. By a number of careful experiments they showed that when infected ticks were fed on healthy monkeys, the latter became infected with the disease and spirochaetes appeared in the blood. They also proved that the tick serves as a true intermediate host for this disease, and that infection is transmitted to their offspring.

Koch (1905), at the same time, investigated the "Tick-Fever" of German East Africa, and made some observations on the life-history of the spirochaete after it had been taken into the gut of the tick. He confirmed Dutton and Todd's observations on the hereditary nature of the infection in ticks, and observed the spirochaetes of an infected tick to make their way from the gut to the ovaries, and bore into the eggs.

Distribution. The Relapsing Fever of Tropical Africa has been recorded from Uganda, the Soudan, British, German and Portuguese East Africa respectively, Nyasaland, Rhodesia, the Congo Free State, the Portuguese Congo and Angola. Recently, a relapsing fever transmitted by ticks and probably identical with the above, has been discovered in Zululand, as far south as the Umphalosi River[1]. Up to the present however, no attempt has been made to collect the records of its distribution and also very few observers have given the exact localities

[1] Unpublished report. Dr C. Hill, Government Medical Officer for Natal, kindly supplied me with this information.

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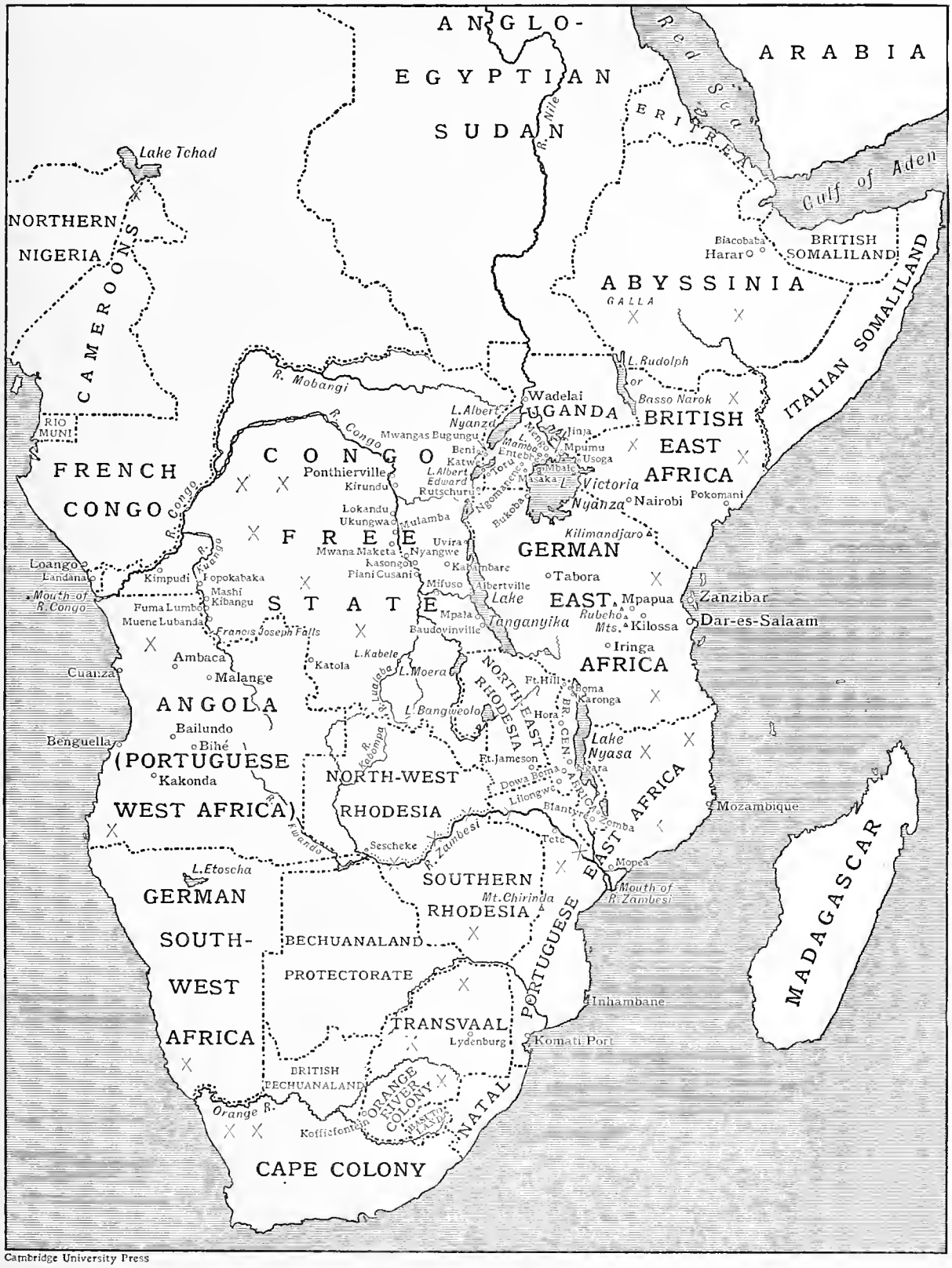
MAP A. The distribution of the Relapsing Fever of Tropical Africa. Only the names of places from which the disease has been definitely recorded are given in this map. Infected States are marked with a cross.



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MAP B. The distribution of *Ornithodorus moubata* (after Merriman). In this map also, only the names of places from which the tick has been definitely recorded are represented.

in which the disease occurs. The following list contains all the places which I have been able to collect, from which the disease has been definitely recorded. In addition these localities are shown in the accompanying Map (Map A) of the distribution of the Relapsing Fever of Tropical Africa. It is hoped that investigators in Africa will give records of the exact localities in which they observe this disease so that it will be possible to see how rapidly it is spreading. There is no doubt that during the last 50 years the disease has spread very rapidly. In 1854 it could not have been common in Angola or else Livingstone (1857) would have noticed it, but although he distinctly mentions the occurrence of *O. moubata* and the effects of its bite, the fever is only mentioned as occurring in Portuguese East Africa, at Tete. At present this disease is common in Angola (Wellman, 1906), probably having been introduced by travellers from the Zambesi. Livingstone (1871) was also annoyed by ticks at Nyangwé but does not mention the occurrence of the fever, whereas in 1904 (Dutton and Todd, 1905) the same locality had a particularly bad reputation for this disease. These and other cases all tend to show that the distribution of this fever has widely extended during recent times, probably as a result of the increased facilities of communication.

The distribution of *O. moubata* is shown in Map B, for comparison with that of the spirochaetosis which it carries. It will be noticed that its distribution is much wider than that of the disease and accordingly there is plenty of opportunity for the latter to spread. It is probable that in a few more years it will occur in most if not all of the regions where *O. moubata* is found, for at present there is so much communication between one state and another that the possibilities of infected ticks being carried from one place to another are very great.

Dutton and Todd note that the ticks occur mostly along the Arab trade routes and have been introduced into the Oriental Province of the Congo Free State from the East Coast. The main centre of the Relapsing Fever of Tropical Africa is probably German East Africa, where Koch (1905) found that a large percentage of ticks from outlying districts were infected, as well as those along the trade routes. The disease is also very prevalent in Uganda, as a result of the communications which have existed between these two countries. The next centre of infection seems to have been the banks of the river Zambesi, which was infected in Livingstone's time (1857). From these two centres the disease has spread along the East Coast of Africa from British East Africa and possibly Somaliland in the north, to Zululand

in the south. Within a few years it spread up along the river Zambesi as far inland as Sescheke, above the Victoria Falls; and from Tete to Nyasaland, which is now heavily infected. It is known that the Arab trade route runs from Tete to Lake Nyasa and along the west side of the lake before going inland to Lake Tanganyika. In the Congo the disease occurs only along the Arab trade routes and especially in the places, *e.g.* Nyangwé, Kasonga, etc., which were used as head-quarters. Dutton and Todd found that in the Congo *O. moubata* collected only a short distance away from the Arab trade routes were uninfected, thus indicating that the disease has only recently been introduced into the Congo along the lines of travel and has not yet had time to spread into the surrounding country, as it has done in German East Africa.

The way in which the disease has been introduced into Portuguese East Africa is not quite so clear but is probably the result of caravans from Tanganyika carrying infected ticks with them and thus establishing the disease wherever these escaped.

With the increased facilities for communication from one state to another, combined with the ease with which *O. moubata* may be carried in bedding, clothes, etc., it seems extremely probable that the Relapsing Fever of Tropical Africa will soon extend into all parts where the tick occurs.

In the following table is given a list of the places from which this disease has been recorded, together with the date of the record and the name of the observer.

Aetiology and Pathology. As mentioned above the Relapsing Fever of Tropical Africa is caused by the presence of *S. duttoni* in the blood of the infected patient. The disease is carried from man to man by the human tick, *O. moubata*, and as far as we know, by no other means, the tick producing infection through the excretion of infective material from the gut, which enters the open wound caused by the tick's bite (Leishman, 1910; Hindle, 1911). The morphology of the parasite together with its life-history in the tick will be reserved for a future communication. The mechanism of transmission, together with all the literature on the subject, has been described in a previous paper (Hindle, 1911).

Symptoms. In man, the incubation period after the tick's bite is usually about a week, but may be prolonged to ten or eleven days. It is believed by some of the natives that when the bite is followed by severe local effects, such as inflammation and swelling, the patient

State	Exact locality	Date	Observer or recorder
Abyssinia	Galla (?)*	1901	Brumpt.
Soudan	... ...	1906	Werner.
British East Africa ...	Kilimandjaro	1901	Brumpt.
	Nairobi	1908	P. H. Ross.
German East Africa ...	Rubeho Mts.	1905	R. Koch.
	Dar-es-Salaam ...	,,	,,
	Kilossa	,,	,,
	Mpapua	,,	,,
	Iringa	,,	,,
	Tabora	,,	Dutton and Todd.
Uganda	Usoga	1903	Christy.
	Wadelai	,,	,,
	Katwe	,,	,,
	Mbale	1907	Simpson.
	Entebbe	,,	,,
	Ngomanene	,,	,,
	Lake Mamba	,,	,,
	Mpumu Chagwe ...	1909	P. H. Ross.
Portuguese East Africa	Tete	1857	Livingstone.
	River Zambesi ...	...	Sir John Kirk.
	Mozambique	1908	Wellman.
	Inhambane	1909	C. W. Howard.
	Lilongwe	,,	,,
	Mopea, Quilimane ...	1911	,,
	Komati Port, Delagoa Bay	,,	,,
Zululand	... ...	1911	Dr C. Hill (personal communication).
British Central Africa	... ...	1900	Daniels.
	Blantyre	1908	Nuttall.
	Fort Hill (N. Nyasa) ...	1909	J. B. Davey.
	Dowa Boma, Angoniland	,,	H. Hearsey.
	Ngara	,,	,,
	Hora	,,	H. S. Stannus.
	Boma, Deep Bay ...	,,	S. A. Neave.
	Karonga	,,	,,
Rhodesia	River Zambesi		Sir John Kirk.
	Sescheke		,,
Congo Free State ...	Kasongo	1905	Dutton and Todd.
	Lokandu	,,	,, ,,
	Mulamba	,,	,, ,,
	Mwana Maketa ...	,,	,, ,,
	Nyangwé	,,	,, ,,
	Ukungwa	,,	,, ,,
	Uvira	,,	,, ,,
	Beni, Lake Albert Edward	,,	,, ,,
Portuguese West Africa	Ambaca	1906	Wellman.
	Bailundo	,,	,,
	Bihé	,,	,,
	Benguella	,,	,,
	Kakonda	,,	,,
	Landana	,,	,,
	Malange	,,	,,

* This record may refer to some other variety of spirochaetosis, and not the Relapsing Fever of Tropical Africa.

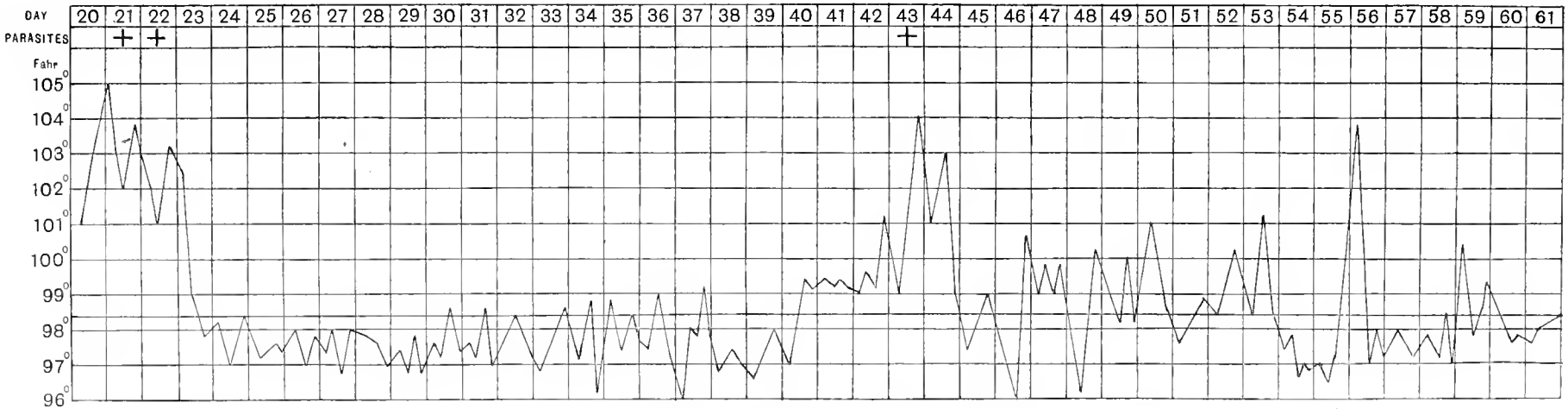

CHART I. G. M. Case of laboratory infection with *S. duttoni*. For particulars refer to text.

usually escapes the fever, and Nuttall (1909) suggests that this may be due to the protective effect of a local reaction.

In the majority of cases amongst Europeans, slight prodromal symptoms, such as mental depression, lack of activity and profuse sweating, often occur a day or two before the onset of the fever. The attack then sets in with headache, vomiting, pain in the back and limbs and severe pain in the spleen. A marked disinclination for food and intense thirst are also noticeable and diarrhoea is often present. The temperature rises rapidly and may reach as high as 104°–105° F., this rise in temperature being accompanied by a feeling of chilliness. The spleen is found to be considerably enlarged, projecting far below the costal margin, and spirochaetes occur in scanty numbers in the peripheral circulation. The attack becomes more severe as the number of parasites increases, and in some cases additional symptoms, such as iritis, may appear. The symptoms generally last from three to four days, and end by a crisis, during which the patient sweats profusely and the temperature falls below normal. There is a marked leucocytosis just before the crisis, and the spirochaetes disappear from the peripheral circulation.

There are usually three or four attacks each lasting about 3–4 days, and during the intervening periods all morbid symptoms disappear. Each relapse begins with a rise in temperature and a return of the original symptoms together with the appearance of spirochaetes in the blood, also oedema of the eyelids is stated to occur in the relapses. The intervening periods between the relapses vary in length from one day up to as long as two months, but are usually about 7–10 days.

Iritis is often observed as a complication.

In connection with the foregoing account of the general symptoms of this disease it may be of interest to describe a somewhat atypical case which occurred in the Quick Laboratory:—

G. M. European, ♂, aged 25. (Chart No. 1.) *Patient's own notes*[1].

The patient was not aware of having been bitten by the ticks but on April 1st had been examining a large supply of infected ticks that had been sent by P. H. Ross from Uganda. Next morning the characteristic marks (3) of the bite were noticed on the forearm.

April 1st. Presumably was bitten on the forearm by three nymphs of *O. moubata.*

[1] My thanks are due to Mr G. Merriman for kindly supplying me with these notes, and also for the temperature chart.

April 19th. Not feeling well.

April 20th. Woke up with severe headache and temperature of 101° F. Pains in back and shoulders as if bruised, muscles of the legs tender, especially in thighs, and weak feeling about knees. Had a hard dry cough which gave great pain in the head and cold chills running up and down the back. About 11 a.m. vomited, and also passed four loose stools. During the day the temperature rose to 104·2° F., and the symptoms became more severe. There was a burning dry heat all over and the soles of the feet felt very tender as if one had walked for miles on hot pavements. At 10 a.m. took Liquor Am. Acet. 2 drachms; Spir. Ethia. Nit. 15 minims; Syr. Pin. Heroin 20 drops; Water A.A. 1 oz., and afterwards felt easier. At midnight the temperature was 105° F.

April 21st. Passed a restless night the cough being troublesome, and felt very weak in the morning though rather easier. There was perspiration and acute pain in the eyes, accompanied by frontal headache.

April 22nd. Spirochaetes were found in the blood in very scanty numbers. At 6 a.m. the eyes were very painful, it being an effort to keep them open. The sight was failing and could only see a short distance with difficulty. At 10 a.m. woke up blind. There was great pain in the head and eyes, and acute photophobia. The eyelids were swollen. Took a Seidlitz powder and passed five loose stools. The cough better.

April 23rd. Still quite blind; head and eyes very painful. Felt very weak. The eyelids were not quite so swollen and the cough practically gone. Temperature fell to normal towards evening.

April 24th. Still blind but pain in eyes better and no headache. Took ½ Seidlitz powder and bowels acted at once.

April 25th. Had a good night and found sight almost returned, getting better during the day. Felt well.

From this date until May 8th there were no pathological symptoms, the patient feeling well and the temperature remaining normal.

May 8th. Passed three loose stools before 10 a.m.

May 9th. Slight rheumatoid pains in the lower extremities.

May 10th. Severe rheumatoid pains in thighs. The eyes ached and were painful to the touch.

May 11th. Pains in legs still continued. The eyes were painful both to touch and to light. Slight huskiness.

May 12th. Previous symptoms continued.

May 13th. Awoke blind, with the exception of being able to distinguish the windows. The eyes very painful and eyelids swollen. Vomited about 6.30 p.m. Severe rheumatoid pains in back and limbs. Cough troublesome. Spirochaetes were found in the blood.

May 14th. The previous day's symptoms continued.

May 15th. Cough almost gone; head better. Blind and deaf.

May 16th. Passed a good night. Still blind, but eyes less painful and cough almost gone.

May 17th. Headache and eyes better; photophobia less.

May 18th. Pain in head and eyes increased towards the evening and rheumatoid pains severe.

May 19th. Awoke with the same symptoms as the previous evening but during the day they became better and the headache and pain in eyes went away. Still blind.

May 20th. Woke up at 6 a.m. to find sight had returned. Eyes very sensitive to light and bad headache. During the day the eyes became less sensitive and vision cleared.

May 21st. Passed a good night. Head and eyes almost well. Temperature normal.

May 22nd. Vomited once and returned to bed.

May 25th. Also vomited about 8 a.m.

No further symptoms were noticed after this date.

One of the interesting features of this case is the extraordinarily long incubation period of 19 days. Moreover, although iritis is a common complication of this disease, acute photophobia accompanied by blindness has not previously been recorded.

From a comparison of the symptoms in natives and Europeans infected in Uganda and German East Africa, P. H. Ross (1907) has distinguished two types of the disease. Dutton and Todd (1905), however, in the Congo found practically no difference between the effects of the disease in Europeans and natives. The milder character of the disease in natives may be explained by the fact that infection has taken place in childhood (Koch, 1906), as most of the children are exposed to infection in the native huts where the ticks occur.

Prognosis. The prognosis for both Europeans and natives is usually favourable, but death may occur, in which case its approach is indicated by a rapid fall of temperature without any improvement of the general symptoms.

The mortality is not more than 5 per cent. under ordinary conditions, but in adverse circumstances it may be much higher. Thus, Dutton

and Todd (1905) mention an instance in which out of twenty men that contracted the disease in one caravan, ten of them died.

Treatment. Up to the present there are no records of any treatment having been successfully used to cure the disease in its natural host, man; but it seems probable that "606" will cure the disease. The following experiments have been made on the treatment of the disease in laboratory animals:—

Vassal (1907) found that the benzidine colours had an effect upon *S. duttoni.* In the case of rats infected with the spirochaete, the injection of certain of these colours, 2–3 days after the inoculation of the disease, was often followed by the disappearance of the parasites within 24 hours. Moreover if the colour was injected together with the spirochaete the course of the disease was always retarded and sometimes prevented.

J. McIntosh (1910) found that the injection of 0·005 gm. of dioxydiamidoarsenobenzol ("606") into each of a number of rats infected with relapsing fever, caused the parasites to disappear within 6–18 hours. Moreover the rats were then immune to any further infection.

Yakimoff and Kohl-Yakimoff (1910) have carefully investigated the treatment of *S. duttoni* in mice and rats. When mice infected with *S. duttoni* were injected with "606" on the first, second, or third day after the appearance of spirochaetes in the blood, the majority of the animals were cured, and in every case the parasites were driven out of the circulation. In a few cases, however, the spirochaetes reappeared in the blood. An injection of "606" administered 24 hours previously to the inoculation of the disease, immunised both rats and mice against infection.

These investigators also found that the injection of benzidine colours into infected animals caused the spirochaetes to disappear for 6–21 days, but then they reappeared. They also found that injections given 24 hours previously to the inoculation of the disease prevented any infection.

In a later paper (1911) Yakimoff and Kohl-Yakimoff confirm their previous results on the treatment of this fever by "606." From their experiments it seems possible that this drug will prove successful in the treatment of this disease in human patients.

Immunity, etc. The fact that recovery from African Relapsing Fever is followed by a certain degree of immunity from further attacks was first noted by Sir John Kirk on the Zambesi, and later by Daniels (Manson, 1903) who observed the disease in Nyasaland.

Breinl and Kinghorn (1906 b) were the first to publish an account of the immunity reactions of *S. duttoni* in experimental animals. They showed that, with the exception of cats, all the common laboratory animals may be infected by inoculations, but that the most susceptible are white rats and monkeys. It was found that after recovery from an infection animals possess a "relatively active immunity against reinfection," as previously shown by Gabritschewsky (1905) in the case of European Relapsing Fever, but that immune serum had no appreciable effect in preventing, or curing, the disease in other susceptible animals. In some cases the incubation period was prolonged when the inoculation had been followed by an injection of immune serum, but the severity of the attack was not appreciably diminished. On the other hand, an injection of hyperimmune serum, besides prolonging the incubation period, mitigated the severity of the infection and occasionally cut short the attacks. They also found that the spirochaete of the Relapsing Fever of Tropical Africa differed from *S. recurrentis* in its immunity reactions, for animals which had recovered from the former were liable to infection with *recurrentis*, and *vice-versa*.

The same authors (Breinl and Kinghorn, 1906 a) showed that *S. duttoni* is capable of passing through the placenta and infecting the foetus; and that the young of an infected mother are born with a slight immunity which is of short duration.

Novy and Knapp (1906) gave a complete account of all previous work on spirochaetal infections and also the results of their own investigations of *S. recurrentis*, especially with regard to its serum treatment on the lines suggested by Gabritschewsky (1905). The results they obtained by treating animals infected with *recurrentis* with hyperimmune serum were very favourable, and led them to make the statement that they had established "a sound basis for the prevention and cure of relapsing fever and the related tick-fever." The results of Breinl and Kinghorn, however, do not support this statement as far as the latter disease is concerned.

Uhlenhuth and Haendel (1907) differentiated the spirochaetes found in European, African and American Relapsing Fevers, respectively. They confirmed the previous observation that infection with any particular strain is usually followed by immunity against the same strain, but not against other varieties. If hyperimmune serum be brought in contact with blood containing spirochaetes of the same strain, a well-marked agglutination takes place and the parasites lose their motility within about half an hour, and undergo granular disinte-

gration. This reaction was found to be specific for each of the three strains investigated and constitutes one of the methods of distinguishing them. They may also be distinguished by means of the Pfeiffer phenomenon. When an animal was inoculated with 0·1 c.c. of hyper-immune serum from a rat or rabbit, and then received 0·5 c.c. of rat's blood containing spirochaetes, if the latter were of the same strain as that used to produce the serum, they disappeared in about 10–30 minutes; whereas, if the spirochaetes belonged to a different strain they persisted for several hours.

Levaditi and Manouélian (1906, 1907) as a result of their researches on this disease, came to the conclusion that the crisis of tick fever is "un phénomène d'ordre purement phagocytaire," thereby resembling that of European Relapsing Fever (Metschnikoff, 1887). During this period the mononuclear phagocytes of the spleen were found to ingest normal spirochaetes. The latter were said to occur only in the blood-stream and no intra-cellular stages were noticed.

Levaditi and Roché (1907) investigated the mechanism of the crises and relapses of this fever, and found that the destruction of the spirochaetes at the crisis was not due to the presence of bacteriolysins or opsonins in the blood, but that these substances seemed to develop some hours later. The relapses were explained on the supposition that the spirochaetes which persist after the crisis, become resistant to the antibodies present in the blood, and then remultiply. According to these authors the resistance to antibodies, acquired at each relapse, is hereditary and therefore cumulative; and after the passage of a particular strain through three mice and two rats they were able to detect a difference in the resistance of the spirochaetes compared with that of the first infection.

Manteufel (1907), however, came to a different conclusion with regard to the mechanism of immunity, and in opposition to the views of Levaditi and Manouélian, showed that the parasiticidal agent is the serum, and that phagocytosis is only a symptom, not the cause of, immunity.

Fraenkel (1907) found that the strain of relapsing fever from East Africa differed in its immunity reactions from that of the Congo, and considered that the two, for the present, must be regarded as distinct. Nuttall (1908) has provisionally proposed the name *S. rossii* for the spirochaete of East African Relapsing Fever, *S. duttoni* being reserved for that of the Congo fever.

Manteufel (1908), in a further account of his investigations on relapsing fevers, showed that the virulence of any particular strain of spirochaetes is very much diminished by passage through a number of experimental animals, *e.g.* rats. He also found that animals which had recovered from a weakened strain of *S. duttoni* did not possess immunity against more virulent strains of the same parasite, but could be reinfected by them.

These observations are of great interest for they show how careful one ought to be in distinguishing spirochaetes by their immunity reactions alone. There is little doubt that Fraenkel's distinction between the East African and Congo strains of spirochaetes respectively may be explained by Manteufel's results, and therefore we propose to regard *S. rossii*, Nuttall, as synonymous with *S. duttoni*, Novy and Knapp. The two strains of spirochaetes are both transmitted, in nature, by the same intermediate host, *O. moubata*, and produce very similar pathological effects in their vertebrate host, man. Moreover it is almost certain, from a consideration of the history and distribution of the disease (see below), that African Relapsing Fever was introduced into the Congo, from German East Africa, within comparatively recent times.

As a further example of the uncertainty of distinguishing spirochaetes by their immunity reactions alone, may be mentioned Darling's (1909) observations on the relapsing fever of Panama. Darling showed that "infection by one strain of spirochaetes is followed by a considerable degree of active immunity for that strain, but such immunity is not potent against another strain from a different source although of the same species and from the same locality, but from a different human host."

Manteufel (1908) also found that *S. recurrentis* may be transmitted by *O. moubata*, and more recently Neumann (1909) has shown that *S. novyi* may also be transmitted by the same means.

Strong (1908) tried to obtain a serum reaction which would enable one to diagnose African Relapsing Fever in the absence of spirochaetes. He found that the precipitin reaction was quite useless for diagnostic purposes, the only suggestion of a reaction being between *S. novyi* and African immune serum.

Tedeschi (1910) has recently published an account of his investigations on the biology of *S. duttoni*. He finds that the immune serum contains agglutinins which are destroyed by a temperature of 58° C.

Opsonins are also developed in very small quantities but the amount of phagocytosis that takes place either *in vivo* or *in vitro* is insignificant.

In connection with the immunity reactions of *S. duttoni* it may be of interest to mention Trautmann's (1907) observations on the effect of spirochaetal infections on the course of the disease of trypanosomiasis. He found that when animals infected with *Trypanosoma brucei, lewisi, equiperdum,* and *gambiense* respectively, were afterwards inoculated with *S. duttoni,* the latter caused the trypanosomes to diminish in number, and often to disappear from the circulation for some time, and consequently the life of the animal was prolonged considerably.

Tedeschi (1910) was unable to notice any effect of *S. duttoni* on the course of the disease in the case of rats infected with *T. brucei* and *T. lewisi* respectively, and consequently throws some doubt on Trautmann's observations.

Recently I have made some observations on the effect of an infection of *S. duttoni* on *T. brucei* and *T. gambiense,* respectively, in mice, and find that the appearance of spirochaetes in the circulation is followed by a diminution in the number of trypanosomes and often the latter disappear for a few days. As a result the length of life of the mice was considerably prolonged in the case of those with a mixed infection, when compared with mice infected with either *T. brucei* or *T. gambiense* alone. These results, therefore, confirm Trautmann's observations.

In conclusion a few words may be added on the present method of distinguishing spirochaetes by their immunity reactions alone.

Darling's observations, mentioned above, show that it is impossible to distinguish them by this method, for in the case of the parasite of Panama Relapsing Fever the spirochaetes from different individuals gave different immunity reactions. Moreover, Manteufel (1908) showed that a particular strain of spirochaetes may become weakened by passage through a series of animals, and then differ in its immunity reactions from the original strain.

It is important to remember that the morphological differences between the various species of blood spirochaetes are insignificant, and at present they are almost entirely distinguished by means of their immunity reactions. Manteufel (1908) and Neumann (1909) have shown that *S. recurrentis* and *novyi,* as well as *duttoni,* may be transmitted by *O. moubata,* and therefore it is impossible to distinguish them by the mode of transmission.

Although the various relapsing fevers of the world produce different pathological effects, yet there are gradations from one form to another, and on the whole it seems probable that all of them are to be regarded as merely local varieties of one widely distributed disease which has become adapted to different intermediate hosts in different localities. Although for the present it is convenient to retain the specific names for the spirochaetes of the various strains of relapsing fevers, yet it is very probable that at the most they are merely varieties of one species of widely distributed spirochaete (*S. recurrentis*).

Prophylaxis. As the disease is transmitted by the bite of *O. moubata*, any prophylaxis is based upon the avoidance of the ticks. If, however, one detects the tick in the act of feeding it should be possible to avoid infection by carefully bathing the tick and the surrounding skin with carbolic solution *before* removing the tick. By this means the infectious excrement of the tick may be sterilised before it has had time to penetrate into the wound caused by the bite.

When travelling through infected country the native huts and rest-houses should never be used, and Koch (1906) advises that Europeans should camp at least 20–30 yards away from any such places.

In order to diminish the number of cases of infection in any district Wellman (1906) advises that the following measures should be adopted:—

The tick should be regularly destroyed in crowded centres by disinfecting native houses, barracks and other permanent quarters, and old camps, huts etc. should be burnt.

Soldiers and other native employees should be made to keep their houses clean and well-swept; they should sleep in hammocks or in beds raised from the floor and away from any wall so that the ticks cannot enter the beds. Natives should never be allowed to sleep near the European quarters.

Soldiers, porters, native servants, etc. should be made to bathe and wash their clothes frequently.

The natural enemies of the tick might be encouraged, for Wellman noticed that certain of the *Reduviidae* fed on them. Moreover they are devoured by rats, and also by many birds.

Also a certain fungus sometimes attacks them with results fatal to the tick.

In conclusion it should be noticed that the Relapsing Fever of Tropical Africa is spreading very rapidly through travellers carrying

infected ticks from place to place in their clothes, bedding, etc. Great care should be exercised by travellers in the choice of camping grounds and also the bedding etc. should be carefully examined for ticks before proceeding to a fresh place.

LITERATURE.

BREINL, A. and KINGHORN, A. (1906 a). The Passage of *Spirochaeta duttoni* from Mother to Foetus. *Lancet*, II, p. 219.

—— —— (1906 b). An experimental Study of the Parasite of the African Tiek Fever. Memoir XXI. *Liverpool School of Tropical Medicine.*

BRÜCKNER und WERNER (1907). Bemerkung zu der Arbeit des Herrn C. Fraenkel : Unterschiede zwisehen den einzelnen Formen des Zeekenfiebers. *Med. klin. Jahrg.* III. 1459.

BRUMPT, E. (1908). Existenee de la "Fièvre des Tiques" en Abyssinie. *Bull. Soc. Path. exot.* I. 432–436.

CASTELLANI and CHALMERS (1910). *Manual of Tropical Medicine.* London. Baillière, Tindall and Cox, pp. 699–711.

CHRISTY (1903). Tiek-Fever in Man. *Brit. Med. Journ.* II. 653.

COOK, A. R. (1904). Relapsing Fever in Uganda. *Journ. Trop. Med.* VII. 24–26.

DARLING, S. T. (1909). The Relapsing Fever of Panama. *Arch. Intern. Med.* IV. 150–185.

DUTTON, J. E. and TODD, J. L. (1905). The Nature of Human Tiek Fever in the Eastern Part of the Congo Free State. Memoir XVII. *Liverpool School of Tropical Medicine.*

FRAENKEL, C. (1907). Unterschiede zwisehen den einzelnen Formen des Zeekenfiebers. *Med. klin. Jahrg.* III. 928–929.

GABRITSCHEWSKY, G. (1905). Zur speeifisehen Therapie der Febris recurrens. *Zeitschr. f. klin. Med.* LVI. 43–48.

HINDE, S. L. (1907). *The Fall of the Congo Arabs.* Cited by Dutton and Todd.

HINDLE, E. (1911). The Transmission of *Spirochaeta duttoni. Parasitology*, IV.

HODGES, A. D. and ROSS, P. H. (1905). Notes on Cases of Spirillum Fever in Uganda. *Brit. Med. Journ.* I. 713–716.

KOCH, R. (1905). Vorläufige Mitteilungen über die Ergebnisse einer Forsehungsreise nach Ostafrika. *Deutsche Med. Wochenschr.* pp. 1865–1869.

—— (1906). Über afrikanische Recurrens. *Berlin klin. Wochenschr.* XLIII. 185–194.

LEISHMAN, W. B. (1907). The Spiroehaete of Relapsing Fever. *Lancet*, CLXXII. 806, 807.

—— (1910). An address on the Meehanism of Infection in "Tick Fever" and on the Hereditary Transmission of *Spirochaeta duttoni* in the Tiek. *Lancet*, CLXXVIII. 11–14.

LEVADITI, C. et MANOUÉLIAN (1906). Reeherches sur la spirillose provoqué par le spirille de la "Tick-Fever." *Compt. Rend. Soc. Biol.* LXI. 566–567.

Levaditi, C. et Manouélian (1907). Recherches sur l'infection provoqué par le spirille de la "Tick-Fever." *Ann. Inst. Pasteur*, xxi. 295–311.

Levaditi, C. and Roché, J. (1907). Les opsonines et le mécanisme de la crise dans la Tick-Fever. *Compt. rend. Soc. Biol.* lxii. 619–621, 815–817.

Livingstone, D. (1857). *Missionary Travels and Researches in South Africa.* John Murray, London, pp. 382–383, 628–629.

—— (1874). *Last Journals.* John Murray, London. iii. 33, 115.

McIntosh, J. (1910). On the influence of the new Ehrlich preparation Dioxydiamidoarsenobenzol ("606"), on recurrent fever in rats. *Lancet*, clxxix. 713.

Manson, P. (1907). *Tropical Diseases.* Cassell and Co. pp. 191–204.

Manteufel (1907). Experimentelle Beiträge zur Kenntnis der Rekurrenzspirochäten und ihrer Immunsera. *Arb. a. d. Kais. Gesundheitsamte*, xxvii. 327–363.

—— (1908). Weitere Untersuchungen über Rückfallfieber. *Ibid.* xxix. 337–354.

Massey, A. Y. (1905). Spirillosis in Portuguese West Africa. *Journ. Trop. Med.* viii. 225.

Merriman, G. (1911). The Geographical distribution of *Ornithodorus moubata. Parasitology*, iv. 168–173, 1 Map.

Metschnikoff, E. (1887). Über den Phagocytenkampf beim Rückfalltyphus. *Virchow's Archiv*, cix. 176–192.

Moffatt, R. V. (1907). Spirillum Fever in Uganda. *Lancet*, clxxii. 208–210.

Nabarro, D. and Greig, E. D. W. (1905). Further observations on the Trypanosomiases (Human and Animal) in Uganda. *Sleeping Sickness Commission, Royal Society*, Report No. V, p. 9.

Neumann, R. O. (1909). Über das Verhalten der Spirochäten des Rückfallfiebers im Tierkörper und die experimentelle Übertragung der Parasiten durch Zecken und Laüse. *München. med. Wochenschr.* lvi. 477.

Nuttall, G. H. F. (1908). Spirochaetosis in man and animals. Harben Lectures, 1908. *Journ. Roy. Inst. of Public Health*, 1908.

Nuttall, G. H. F. and Warburton, C. (1908). *Ticks. Part I.* Cambridge University Press.

Ross, P. H. (1907). Africar Relapsing Fever; in Allbutt and Rolleston's *System of Medicine*, Vol. ii. Part i. p. 381.

Ross, P. H. and Milne, A. D. (1904). Tick fever. *Brit. Med. Journ.*, 1904, ii. 1453–1454.

Schilling, G. (1906). Rückfallfieber, in Mense's *Handbuch der Tropenkrankheiten*, iii. 668–688.

Tedeschi, A. (1910). Experimenteller Beitrag zur Erforschung der Spirochäte des afrikanisches Rekurrensfieber (*Sp. duttoni*). *Centralbl. f. Bakt. u. Parasit.* liv. 12–21.

Trautmann, R. (1907). Étude expérimentale sur l'association du spirille de la Tick-fever et de divers Trypanosomes. *Ann. Inst. Pasteur*, xxi. 808–824.

Uhlenhuth, P. und Haendel (1907). Vergleichende Untersuchungen über die Spirochäten der in Africa, Amerika und Europa vorkommenden Rekurrenserkrankungen. *Arb. a. d. Kais. Gesundheitsamte*, xxvi. 1–10.

Vassal, J. J. (1907). Action des couleurs de benzidine sur le spirille de la "Tick-Fever" (*Sp. duttoni*). *Compt. rend. Soc. Biol.* lxii. 414.

WELLMAN, F. C. (1905 a). Case of relapsing fever, with remarks on its occurrence in the tropics and its relation to tick-fever. *Journ. Trop. Med.* VIII. 97–99.

—— (1905 b). Notes on the Tropical Diseases of the Angola Highlands. *Amer. Soc. Trop. Med.* I. 1–49.

—— (1906). Human trypanosomiasis and spirochaetosis in Portuguese South-West Africa, with suggestions for preventing their spread in the Colony. *Journ. of Hygiene*, VI. 237–245.

WERNER (1906). Zur Epidemiologie des afrikanischen Recurrens. *Arch. f. Schiffs- u. Trop.-Hyg.* X. 776–781.

YAKIMOFF, W. L. and KOHL-YAKIMOFF, NINA (1910). Contribution à la chimiothérapie de la tick-fever avec "606" et la couleur de benzidine. *Ann. Inst. Pasteur*, XXIV. 826–831.

—— —— (1911). Nouvelle communication relative au traitement de la maladie du sommeil et de la tick-fever par le "606." *Bull. Soc. Path. exot.* III. 141.

AERIAL CONTAMINATION AS A FALLACY IN THE STUDY OF AMOEBIC INFECTIONS BY CULTURAL METHODS.

A PRELIMINARY NOTE.

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With Plate VI.

CONTENTS.

I. Introduction.

Dysentery is usually classified as (i) bacillary, and (ii) amoebic.

In 1898 Shiga described a bacillus (*B. dysenteriae*, also known as Shiga's bacillus), as the cause of epidemic dysentery in Japan. Since then his observations have been, in the main, confirmed. Other varieties of dysentery bacilli have been described and "Bacillary Dysentery" seems now to be a well-defined disease capable of study by ordinary bacteriological methods.

With regard to amoebic dysentery, on the other hand, much confusion and radical difference of opinion still exists. Indeed the causal rôle of amoebae in so-called amoebic dysentery does not seem to be finally established (Strong, 1907; Tanaka, 1910).

A form of dysentery associated with the presence of motile amoebae in the stools had long been recognised, but the work of Schaudinn (1903) first gave definition to the subject and indicated lines of research which have since been followed by several other observers. He

distinguished two types of intestinal amoebae (i) *Entamoeba coli,* a harmless commensal and (ii) *E. histolytica,* identified by him with that previously described and figured by Jürgens (1902) as the causal organism in three cases of dysentery originating in China. Schaudinn gave it as his opinion that cultures of parasitic amoebae were not obtainable. He insisted on the necessity of consecutive study of the morphology and life-cycle of parasitic protozoa in their ordinary modes of life. He therefore based the differentiation of these two types on the features as observed in the natural surroundings of the amoebae, i.e. the intestinal contents and also, in the case of *E. histolytica,* in the intestinal walls of men who had died of dysentery and of experimentally infected cats.

Viereck (1907), Hartmann (1908), and Werner (1908), following Schaudinn's methods of observation, described another species of amoeba in the stools from cases of dysentery, *E. tetragena,* allied to *E. histolytica.* Viereck (1907) considered that the natural occurrence of pathogenic amoebae in water had not been convincingly demonstrated and that such amoebae had not been proved to multiply on artificial media; on the contrary, he emphasised the fact that amoebae in stools appear to die off rapidly after their discharge from the body. Werner (1908) attempted to obtain multiplication of parasitic amoebae by transferring to fucus-agar-medium fresh stools containing vegetative forms of *E. histolytica* and *E. tetragena*; in no case did he see multiplication of these amoebae (the number of observations is not stated); on the other hand he found, on the agar medium, growth and encystment of what was apparently a different species of amoeba; this organism he was inclined to identify with *Amoeba limax* (Vahlkampf), a free living form of wide distribution. He showed that, when cysts of *A. limax* were ingested by house-flies, such cysts passed through the alimentary canal apparently unchanged, so that they germinated when the faeces of the infected fly were transferred to a suitable medium such as the agar used in his experiments. Werner suggested that the amoebae appearing in cultures from human faeces might have had a similar history.

In marked opposition to the findings of the above observers are those of Musgrave and Clegg (1906, 1907), Lesage (1905), Walker (1908), and Noc (1909).

In 1904 Musgrave, and in 1906 Musgrave and Clegg, using an agar medium with an alkalinity of 1 %, cultivated amoebae from the stools in cases of dysentery in Manilla, from water, soil and a variety of

outside sources. They did not discover, in any of these amoebae from different sources, characters which would serve to distinguish different species among them, or finally to distinguish these cultivated forms from those found microscopically in the infected human intestine and liver. They considered it probable that any free-living amoeba might, under certain conditions, become pathogenic.

Lesage (1905) stated that he was able to obtain, in seven out of 20 cases of tropical dysentery, cultures of amoebae from the stools, using washed gelatin as a medium. These amoebae he considered as identical with *E. histolytica* Schaudinn. Walker (1908), using Musgrave's medium, obtained from the faeces of different animals 44 cultures of what he considered parasitic amoebae, among which he distinguished ten species; he did not succeed in obtaining a culture from human faeces. Noc (1909) by the use of a gelatin medium, 0·5 % alkaline in reaction, obtained cultures of amoebae from (i) liver abscess pus, (ii) the stools in cases of dysentery and (iii) the water supply of Saigon (French Indo-China): these amoebae he considered to belong to one single species, identical with that found microscopically in the faeces and in liver abscess pus and he looked upon them as the causal organisms of amoebic dysentery and hepatitis.

In the medical literature on amoebae, as will be clear from the above short résumé, opinion is sharply divided on one main issue, as to whether parasitic amoebae have been cultivated on artificial media or not; for the affirmative Musgrave and Clegg, Lesage, Walker, and Noc, for the negative, Jürgens, Schaudinn, Viereck, Hartmann and Werner are quoted above.

The following are contributions to the question from the point of view of the Protozoologist: Vahlkampf (1905) made a study of *Amoeba limax*, a free living organism recovered by him from an infusion of straw in tap water. Werner is inclined to identify the amoebae cultivated by Musgrave with this saprophyte. Vahlkampf mentions that Kartulis cultivated a "Dysentery" amoeba in diluted rabbit's and pigeon's faeces contained in open vessels, and that Kruse and Pasquale considered that these amoebae were not parasitic but were simply saprophytes which had gained access to the cultures from the air.

Nägler (1909), in introducing observations on the life-cycles of some saprophytic amoebae, says "In medicine also an exact knowledge of the life-cycles of amoebae is of great importance; it precludes such error as has arisen in the work of Musgrave and Clegg, for example; these authors have cultivated forms resembling *Amoeba limax* and have given

them out as dysentery amoebae, whereas these cultivated forms have absolutely nothing to do with genuine parasitic amoebae but belong to the *Limax* group."

Doflein (1909), after considering the various amoeboid organisms which have been described as occurring in the faeces, dwells upon the difficulty of coming to any clear idea as to which of the following headings such organisms should come under: (i) "gelegentliche Passanten des Darms," i.e. casual visitors in the gut or pseudoparasites; this group includes saprophytic amoebae swallowed in the encysted condition and excreted unchanged, (ii) facultative parasites, (iii) amoebae specifically adapted to the parasitic habit.

Recently, Whitmore (1911) has examined two cultures, on Musgrave's medium, of amoebae originating in Manilla, one from faeces, the other from liver abscess pus. He came to the conclusion that the amoebae from these different sources showed some differences among themselves but that both belonged to the "*Limax*" group. He supports Hartmann's view that these amoebae have nothing whatever to do with the parasitic amoebae (*E. histolytica* and *E. tetragena*).

I conclude this introductory sketch of the literature on the subject by a reference to a paper by Major W. G. Liston, I.M.S., as yet unpublished, which he has kindly allowed me to read in manuscript. In a culture from liver abscess pus Liston distinguished two species of amoebae and I am deeply indebted to him for the suggestion, among many others, that the forms seen in the cultures now under consideration must, in all probability, represent more than one species.

II. Technique and material of observation.

The medium[1] used in the experiments now recorded was that of Musgrave (1904). The melted agar was poured into small Petri dishes which were left partially open to the air for a few minutes until the medium had "set." Of the plates thus prepared some were directly closed, inverted and placed out in various parts of the Hazaribagh Central Jail building; others were at first left open, with the agar surface completely exposed to the air for varying periods before being closed and inverted.

[1] Agar 20
Sodium chloride ... ... 0·3—0·5
Beef extract ... ... 0·3—0·5
Distilled water 1000
The reaction of the medium is 1 % alkaline.

On all of these plates colonies of moulds and bacteria from the air made their appearance after 1–6 days. The colonies were examined under the low power of the microscope in the inverted plates and, in 14 out of 36 plates, amoebae were found present, in association with one or more of the bacterial colonies. The details of the experiments are tabulated on pp. 210, 211.

Subcultures of these amoebae were readily obtained by taking a loopful of the mixed growth of amoebae, either vegetative forms or cysts, and bacteria, and stroking it on the surface of a fresh plate of Musgrave's medium. The subcultures were generally kept in a moist atmosphere at a temperature of 22°–28° C. for the first day or two: the optimum temperature for their multiplication probably lies within these limits. Generally, after from 12 hours to two days, there is an abundant crop of motile amoebae along the needle-stroke of inoculation; as a rule, after 5–6 days many of the amoebae are found to have become encysted.

In order to obtain a culture which would certainly represent only one species of amoeba, the following method was adopted: A loopful of growth is inoculated at one point of the plate; thence a radiating stroke is produced on the surface of the medium with a fine platinum needle. On examining this stroke under the low power several amoebae are generally found at the end of it. From this point a second stroke is made, and so on, until finally one is selected which contains only a single amoeba towards its extremity: this single amoeba is then cut off from the rest of the growth by a stroke of carbolised vaseline painted on the surface of the medium after the method of Walker (1908). Pure cultures thus obtained were most easily preserved from contamination by subculturing on test-tube "slopes" of Musgrave's agar.

With a Zeiss AA lens and a No. 12 eyepiece the main processes of the life-cycle may be roughly followed on the inverted Petri plates, but, for examination under the higher powers of the microscope, the following method of subculture was employed: a slab of Musgrave's agar, half an inch square, is cut out of a fresh plate of the medium with a sterile knife and placed on a sterile slide. A loopful is then taken from an old culture containing cysts and stroked on the surface of the agar square; a sterile cover glass is placed on top and its edges are sealed with melted paraffin wax. An air space should be left between the edges of the agar slab and the paraffin wall. Thus a microscopic moist chamber is provided, sealed against external contamination. Such a preparation may be kept in a warm microscope chamber at a tempera-

ture of 25° C. Examining such a slide culture under the low power, one picks out a spot towards the tail end of the inoculation stroke where the cysts are thinly scattered; a single one is then isolated under the oil-immersion lens for continuous observation.

For permanent preparations fixation after the method of v. Wasielewski and Hirschfeld (1909) gave the best results. The principal fixatives used were osmic acid (2 %) and corrosive-acetic-alcohol. followed by Giemsa's stain and iron-haematoxylin-eosin respectively. The first method of fixation gives the best picture of the plasma, the second brings out the nuclear material particularly well.

III. Morphology and life-cycle of the amoebae.

A reference to the table shows that, in 12 out of the 14 growths of amoebae obtained from the air in the manner described, two types of cysts were distinguished. The following description is concerned only with the growths of amoebae occurring on one single plate (No. 24 with table).

Cysts of type (*a*) (Pl. VI, fig. 1), have a diameter of 8–14, generally 10–12 μ; their shape is round, oval, more frequently trihedral, polygonal or stellate; the wall is about 0·25 μ in thickness and has a strongly marked double contour; the outer layer is generally wrinkled, reminding one of the outline of an *Ascaris* ovum. The plasma is compact and coarsely granular and a circular or oval eccentric nucleus is, as a rule, more or less distinctly visible in unstained specimens.

Cysts of type (*b*) (Pl. VI, fig. 19) on the other hand, are much smaller, having a diameter of 3–8 μ; the shape is round or oval, with little or no tendency towards the modified forms often assumed by cysts of type (*a*); the surface is smooth and the wall delicate with a faint double contour. The plasma is hyaline with thinly scattered highly refractile granules; a nucleus is hardly to be distinguished.

Cysts of both types stain an intense purple black with iron-haematoxylin.

In order to determine whether these two well defined types of cysts represent two different species or simply polymorphic forms belonging to a single species, attempts were made to obtain cultures originating from a single cyst of each type by Walker's method, as above described. In many cases however the cysts isolated failed to germinate; it was therefore found more practicable to select a single motile individual as the starting point of a strain.

Serial No.	Date	Conditions of experiment	Presence of amoebae	Remarks
1	12th Aug./10	Uninoculated Petri plate left out on laboratory table without preliminary exposure to air	+18th Aug./10	Cysts of types (*a*)&(*b*).
2	12th Aug.	Same as above	– up to 5th Sept.	Medium overgrown by moulds.
3	12th Aug.	Uninoculated Petri plate left out on laboratory table without preliminary exposure to air	– up to 5th Sept.	,, ,, ,,
4	18th Aug.	Plate first opened exposed to the air for 5 minutes then left out on laboratory table as before	– up to 11th Sept.	,, ,, ,,
5	18th Aug.	Similar to above but preliminary exposure to air 10 minutes	– up to 5th Sept.	,, ,, ,,
6	18th Aug.	Similar to above but preliminary exposure to air 20 minutes	– up to 5th Sept.	,, ,, ,,
7	18th Aug.	Similar to above but preliminary exposure to air 30 minutes	– up to 5th Sept.	,, ,, ,,
8	18th Aug.	Similar to above but preliminary exposure to air 1 hour	– up to 5th Sept.	,, ,, ,,
9	18th Aug.	Similar to above but preliminary exposure to air 1½ hours	+22nd Aug.	—
10	18th Aug.	Similar to above but preliminary exposure to air 2 hours	– up to 5th Sept.	Medium dried up.
11	18th Aug.	Plate left out on window-sill of Hospital Dysentery Ward without preliminary exposure to air	– up to 9th Sept.	Medium overgrown by moulds.
12	18th Aug.	Similar to above	– up to 6th Sept.	,, ,, ,,
13	18th Aug.	Plate left out on window-sill of Hospital General Ward without preliminary exposure to air	+23rd Aug.	Cysts of types (*a*) & (*b*).
14	18th Aug.	Similar to above	+28th Aug.	,, ,, ,,
15	18th Aug.	Plate left out on table of Hospital Office without preliminary exposure to air	+22nd Aug.	,, ,, ,,
16	18th Aug.	Plate left out on floor of "Post Dysenteric Gang" Ward without preliminary exposure to air	– up to 11th Sept.	Medium overgrown by moulds.
17	18th Aug.	Plate left out on floor of No. 1 Jail ward without preliminary exposure to air	+30th Aug.	Cysts of types (*a*)&(*b*).
18	18th Aug.	Plate left out in central tower of Jail without preliminary exposure to air	– up to 9th Sept.	Medium dried up.
19	18th Aug.	Plate left out on window-ledge of tank room without preliminary exposure to air	– up to 6th Sept.	Medium overgrown by moulds.

Serial No.	Date	Conditions of experiment	Presence of amoebae	Remarks
20	18th Aug.	Plate left out on table of Jail Office without preliminary exposure to air	– up to 6th Sept.	Medium overgrown by moulds.
21	12th Dec.	Uninoculated Petri plate left out on laboratory table without previous exposure to air	+3rd Jan./1911	Cysts of types (*a*) &(*b*).
22	12th Dec.	Petri plate first opened and exposed to the air for 5 minutes, then left out on laboratory table as before	– up to 3rd Feb.	Medium dried.
23	12th Dec.	Similar to above but preliminary exposure to air 10 minutes	– up to 3rd Feb.	,, ,,
24	12th Dec.	Similar to above but preliminary exposure to air 20 minutes	+3rd Jan.	Cysts of types (*a*)&(*b*).
25	12th Dec.	Similar to above but preliminary exposure to air 30 minutes	+3rd Jan.	,, ,, ,,
26	12th Dec.	Similar to above but preliminary exposure to air 1 hour	+3rd Jan.	,, ,, ,,
27	12th Dec.	Similar to above but preliminary exposure to air 1½ hours	+3rd Jan.	,, ,, ,,
28	12th Dec.	Similar to above but preliminary exposure to air 2 hours	+13th Jan.	,, ,, ,,
29	12th Dec.	Petri plate left out on window-sill in Hospital	+3rd Jan.	,, ,, ,,
30	12th Dec.	Petri plate left out on window-sill in Hospital General Ward, without preliminary exposure to air	– up to 3rd Feb.	Medium dried.
31	12th Dec.	Petri plate left out on table iu Hospital Office without preliminary exposure to air	+6th Jan.	Cysts of types (*a*)&(*b*).
32	12th Dec.	Petri plate left out on floor of P. D. Gang Ward without preliminary exposure to air	– up to 3rd Feb.	Medium dried.
33	12th Dec.	Petri plate left out on floor of No. 1 Ward Jail without preliminary exposure to air	– up to 3rd Jan.	,, ,,
34	12th Dec.	Petri plate left out on ledge near top of Central tower of Jail, without preliminary exposure to air	– up to 3rd Jan.	,, ,,
35	12th Dec.	Petri plate left out on window-ledge of tank room without preliminary exposure to air	– up to 3rd Jan.	,, ,,
36	12th Dec.	Plate left out on table of Superintendent's Office without previous exposure to air	– up to 3rd Jan.	,, ,,

(1) *Amoeba of type (a).*

In this way cultures of *Amoebae* were obtained which were found to form cysts of type (*a*) only. If such a culture be allowed to encyst, the cysts be transferred to a slide preparation, as above described, and one of them be isolated under the 1/12th immersion-lens, its development may be followed in detail.

After 1–3 hours (at a temperature of 22°–28° C.) a faint streaming movement of the contained granules becomes evident and a bright vacuole appears in the plasma. After a very variable period—from 10–35 min.—the vacuole suddenly shuts and either disappears entirely or leaves only a minute dark speck to mark its site; then, very slowly and gradually, it reappears. This process is repeated at diminishing intervals until the vacuole may contract after irregular periods of 40–180 seconds, the streaming of the granules becoming more and more active and the nucleus more clearly apparent in the meantime. After 3–7 hours, as a rule, from the time of insemination, during active movements of the contents, a small knob of protoplasm is seen to thrust suddenly through the cyst wall at one point (Pl. VI, fig. 2). Streaming through the narrow outlet, the active amoeba rapidly increases in size, so that a considerable portion of it may escape without any coincident shrinking of the contained part away from the inner wall of the cyst. The amoeba may become completely clear of the cyst within 5–20 minutes of the rupture, and may, by that time, have reached a diameter of 15–25 μ.

The *nucleus* stands out distinctly as a circular or oval body with a dark centre and a clear peripheral halo; the total diameter is about 2·5 μ. One *contractile vacuole* is invariably present which, at this stage, generally contracts, somewhat irregularly, once in 20–80 seconds and there may, more rarely, be two or more; clear circular areas, about 1–2 μ in diameter, which remain uncontracted are often seen in the plasma (Pl. VI, fig. 5). Sometimes the distinction between ectoplasm and endoplasm is remarkably clear but often the protoplasm is uniformly granular. In this respect the same amoeba shows varying characters at different times. The *pseudopodia* vary much in form, may be broad and blunt or spinous, simple or branched; the commonest form seems to be a single, rather broad or blunt process, the margin of which is fringed with short spikelets. Several pseudopodia may be protruded in different directions at the same time; many bacteria may be in-

gested. In specimens fixed with osmic acid and stained by Giemsa's solution the blunter pseudopodia are well demonstrated (Pl. VI, fig. 14).

After its escape from the cyst the amoeba continues to move more or less actively over the surface of the fresh medium, leaving a trail of bacteria in its wake. Actively motile amoebae of this type have been watched on 12 occasions for periods of from 1–6 hours without showing any radical change in their behaviour. On the other hand, on nine occasions, an amoeba under examination was observed to shrink and assume a rounded or oval form (Pl. VI, fig. 6), then the body undergoes an hour-glass constriction and finally divides into two daughter amoebae, each approximately half the size of the parent (figs. 7 and 8); the whole process from the first evidence of constriction till complete division takes about two minutes.

The process of nuclear division was not followed in fresh specimens; indeed I have found the behaviour of these amoebae, while undergoing fission, to be in this respect similar to that observed by Liston in one strain of amoebae isolated by him from liver abscess pus. As in the amoeba described by Liston, on the condensation of the plasma previous to division, the nucleus becomes obscure, nor in the new daughter amoebae is a nucleus immediately to be seen; it takes shape gradually and not till 2–5 minutes after division does it become clearly visible.

In stained specimens, some amoebae were seen in which the nucleus, while taking the stain deeply, showed an appearance suggestive of simple fission (Pl. VI, fig. 15); in none was evidence of karyokinesis seen. In one stained specimen (fig. 16), an amoeba was seen showing an hour-glass constriction in the middle with a clear vacuole at each pole, without distinct nuclear staining.

In fresh specimens, on many occasions a small portion of a motile amoeba was seen to become detached, these fragments showed no active movement and ultimately disappeared from view after half an hour or more. On four occasions however true motile buds, 2–5 μ in diameter, were seen to emerge from the parent body.

In specimens fixed by corrosive-alcohol-acetic acid and stained by iron-haematoxylin, an amoeba is often seen to contain one to 12 or more clear rounded spaces 1 to 4 μ in diameter, with or without a central or eccentric dot which takes the chromatin stain faintly (figs. 17 and 18); these apparently correspond to the "internal buds" or "merozoits" of Noc.

Many small protoplasmic masses, one or more μ in diameter, some containing a distinct nucleus, others without any chromatin staining,

are also seen in stained films from the cultures, sometimes collected into groups. The small bodies without any visible chromatin possibly represent the detached fragments of protoplasm seen in fresh specimens. It is, therefore, evident that multiplication by unequal budding also takes place in this amoeba. A third striking modification in the activity is very commonly seen in these cultures. An amoeba may throw out highly refractile globular or blunt branched motile processes 2–4 μ in diameter at one or more points on its surface. The whole of the original body may in a few minutes be completely overlaid by or absorbed in these amoeboid excrescences which adhere to one another, the original nucleus being entirely lost to view. Thus a writhing lobulated mass is produced which may assume the most bizarre forms, moruloid, moniliform or cochleate; the lobules composing it, some of which often show contractile vacuoles, may lie one on top of the other, so that all cannot be focussed at once (figs. 11–13). This process has been noted to occur in more than 12 cases while individual amoebae were under observation. Such bodies have been consecutively observed under the higher powers of the microscope on 20 recorded occasions with the following results: (i) in four cases, the lobulate body has moved about for periods of from 35 min. to 5 hours and changed its shape continuously but has not undergone any alteration in character. (ii) In 11 cases one of the elements composing the mass has been seen to absorb the others into itself, gradually acquiring a visible nucleus, until an amoeba of the ordinary vegetative type results. This amoeba was observed (*a*) in three cases to retain its characters unaltered for periods up to 2 hours, (*b*) in five cases to reassume the lobulate form after periods of from 3 min. to $1\frac{1}{2}$ hours, (*c*) in one case to give off a bud, 5 minutes after formation, (*d*) also, in one case to divide into two, 3 hours 15 minutes after formation. (iii) In two cases the lobulate body has been observed to divide into two similar masses. (iv) In three cases one of the processes has been observed to become detached, acquiring a visible nucleus and all the characters of the usual vegetative type. No forms definitely corresponding to these lobulate bodies have been found in stained specimens.

The significance of this form of activity is not clear; it may perhaps represent either a modification of the process of budding or a third distinct method of reproduction by multiple division[1].

[1] Liston informs me that he has observed similar appearances in his cultures and that he attributes them to the fact that the amoebae concerned "are simply penetrating the

Three or four days after insemination some of the amoebae generally become encysted. Encystment seems often to be immediately preceded by a remarkably active multiplication; this results in the formation of clumps of amoebae which may become massed together, giving the appearance of plasmodia. In the end each amoeba becomes rounded, the body shrinks, becomes more granular in appearance and acquires a thin envelope; this is at first single but later presents the characteristic appearance of double contour, the outer layer being wrinkled (figs. 12 and 19). The original circular outline may be modified to become trihedral, polygonal or stellate, the nucleus becomes less distinct, the contractile vacuole ceases to open, until it becomes apparent that the youngest generation has reached the cystic stage from which its ancestors set out. In stained specimens many of the amoebae undergoing encystment are seen to contain numerous internal buds (fig. 19). Not all the amoebae encyst about the same time; indeed, many are often found still motile in cultures more than a month old.

(2) *Amoeba of type (b).*

Attempts to obtain a culture the progeny of a single individual of this type did not succeed, but ultimately a culture originating from a number of small motile forms was obtained which in subculture was invariably found to yield cysts of the small type only.

Germination of the cyst has not been directly observed in this strain; on six occasions a single cyst on fresh medium was kept under the oil-immersion lens for from 10 hours to three days without showing any signs of germination. Empty cyst shells have not been seen in unstained specimens, but in stained films from the cultures they appear as rounded shrivelled bodies about 3–5 μ in diameter (fig. 24). With

surface of the agar." He points out that amoebae grow below the surface of the agar, and that, in old cultures cysts may be focussed in various planes of the medium.

I find that this is apparently a constant phenomenon in cultures of type (*a*).

It is very difficult to interpret the active movements of these burrowing amoebae, as different points of their surface are at different levels, so that the whole body cannot be focussed at one time. Moreover, another amoeba might suddenly come into view in the field of the microscope having entered it from a higher or lower flow and thus give rise to an appearance of division on the part of the amoeba originally under observation. It seems at least probable that the activities of these lobulate bodies here described may be due to modified processes of fission and budding going on below the surface of the agar. The fact that these forms occur only below the surface accounts for their absence in fixed and stained films.

Giemsa's stain they take a brownish or greenish colour, with iron-haematoxylin they are light yellow.

The vegetative amoebae of this type seldom measure more than 12 μ. Many small forms are also found, measuring as little as 2 μ. The vegetative form (fig. 21) is generally very filmy and delicate, showing a faint hyaline plasma containing scattered granules without any definite distinction between ectoplasm and endoplasm. Pseudopodia are commonly single and rather broad and blunt; spinous pseudopodia were not seen in this form. The nucleus is generally hard to distinguish in unstained specimens but may appear as a faint pinkish circular area 1–2 μ in diameter. One contractile vacuole is generally present in the active amoeba and contracts once in 25–70 seconds. Movement is, as a rule, more active than in type (*a*), the whole body gliding rapidly, often without any appreciable formation of pseudopodia. Fission has been observed in fresh specimens 24 times (figs. 22 and 23). The amoeba does not lose its motility and become condensed before fission as in type (*a*) but while actively motile it suddenly assumes a dumb-bell shape and divides into two daughter amoebae within one minute. The formation and detachment of a bud about 1–4 μ in diameter have been observed in fresh specimens on four occasions. Nothing has been observed in this strain approaching the formation of the lobulate bodies which are so characteristic in cultures of type (*a*), but, on the other hand, the following curious modifications of activity have been noticed.

An active amoeba may suddenly become constricted in the middle, the two halves separating and giving a transitory appearance of fission: they, however, immediately come together again, the amoeba resuming its former habit. In the same way a small portion of the amoeba may appear to separate off from the main body to become immediately reabsorbed. These appearances are suggestive of abortive fission and gemmation respectively. On two occasions in cultures of this type, a motile amoeba has been observed to become spherical and acquire a double contoured envelope. One of them was a daughter amoeba the product of fission one hour old when encystment commenced; in the other case the amoeba had given off a bud 35 minutes before.

In stained specimens the vegetative forms have a diameter of 2–14 μ; the nucleus, which in a well grown form measures about 2 μ, stains less intensely than in the amoeba of type (*a*); osmic acid fixation followed by Giemsa staining fails to show a clear distinction between ectoplasm and endoplasm (fig. 25). Fission forms (fig. 27) and forms containing endogenous buds (fig. 26) are seen, as also vacuolated bodies without

any well defined nucleus but with more or less scattered irregular chromatin masses or granules.

The details of the life-cycle of these two types of amoebae have been studied in cultures on solid agar only; however both types (*a*) and (*b*) have been found to multiply to some extent in diluted broth (1 in 10, 1 in 100), though this medium does not seem to be at all so favourable to their growth as is Musgrave's agar.

Flagellated forms, such as von Wasielewski and Hirschfeld found to develop in growths of "straw amoebae" transferred from agar to weak broth, were not observed in either type. In the fluid cultures no contractile vacuole was observed in the vegetative phase. The cysts were characteristic of their respective types and similar to those found in cultures on agar.

IV. Conclusions.

1. Amoebae of at least two different types are, in this part of India at any rate, commonly present in the air, just as are many moulds and bacteria.

2. These amoebae can readily gain access, (i) to specimens of faeces, however carefully collected, (ii) to specimens of pus or other material which has, either before or after removal from the body, been exposed to the air and (iii) to any material after it has been inseminated on Musgrave's medium contained in Petri dishes.

These facts indicate yet another source of confusion in dealing with cultures of amoebae, from faeces, in addition to those mentioned by Doflein (1909) as quoted in the Introduction (p. 207).

In view of the confusion which at present obtains in the classification of amoebae, no attempt is here made to assign the two organisms described to any particular species.

However, the morphology and life-cycle of these undoubted saprophytes have, at least, enough in common with the features described by a large group of authors[1] as characteristic of true parasites to give rise to serious confusion.

I wish to express my gratitude to Major A. F. Stevens, I.M.S. Superintendent, Central Jail Hazaribagh, for his kindness in providing every facility for this work in the Jail and for many valuable suggestions.

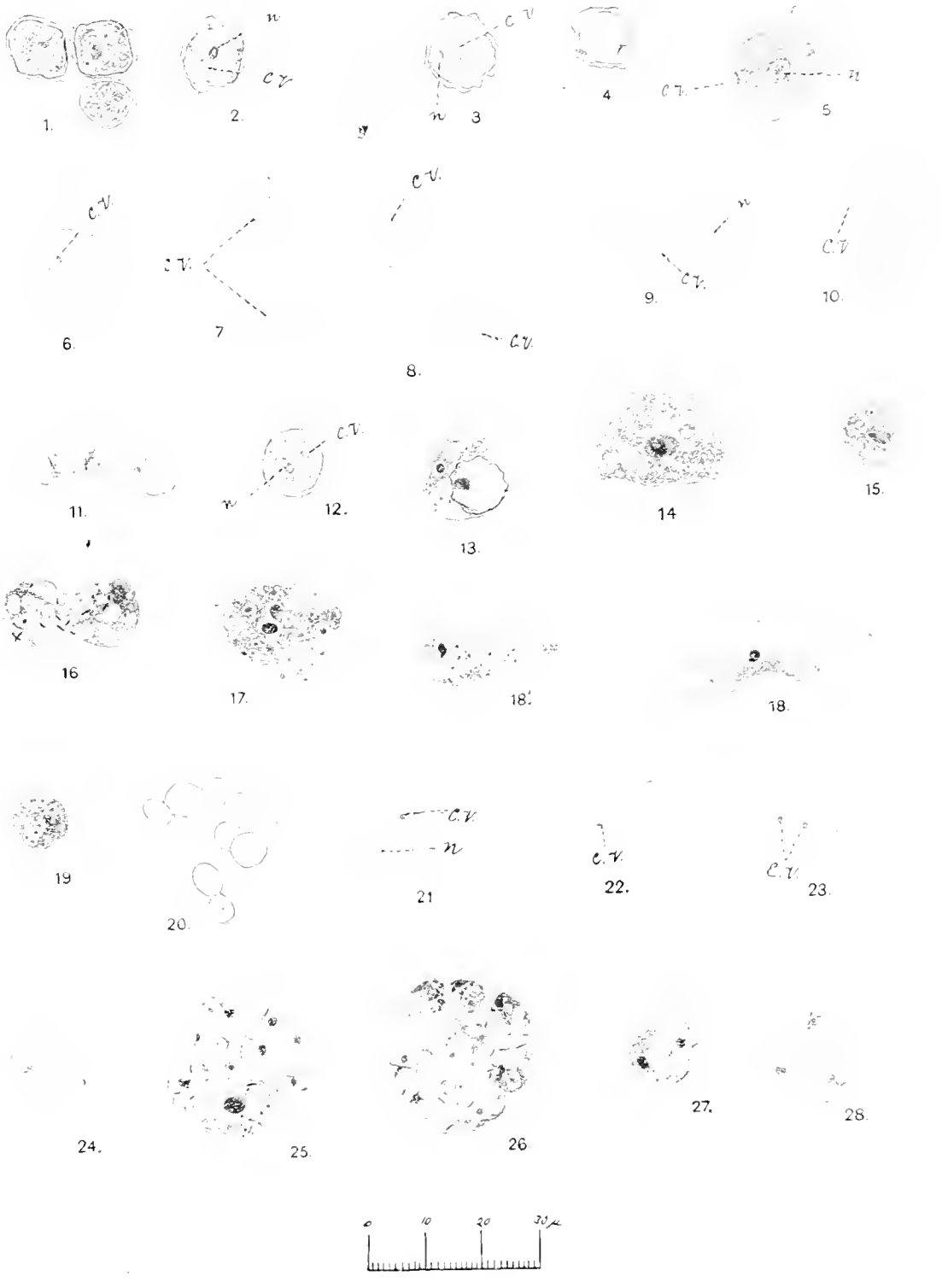
[1] Musgrave and Clegg, Nägler, Noe, Schaudinn, Strong.

I would also acknowledge my indebtedness to Major W. G. Liston, I.M.S., who very kindly gave me much helpful advice by correspondence, acting upon which I have undertaken the differentiation of the two types of amoebae and have put the subject in its present form.

I should like, finally, to record my appreciation of the very ready help given me by Sub-Assistant Surgeon, Jugesh Chandra Guha in these observations.

REFERENCES.

Doflein (1909). *Lehrbuch der Protozoenkunde*, p. 514.—Hartmann (1908). Eine neue Dysenterie Amöbe. *Beihefte z. Arch. f. Schiffs- u. Tropen-Hygiene*, xii. No. 11.—Jürgens (1902). Zur Kenntniss der Darmamöben und der Amöben-Enteritis. *Veröffentl. a. d. Geb. d. Milit. Sanitätswesens*, xx. 110.—Lesage (1905). Culture de l'amibe de la dysenterie des pays chauds. *Ann. Inst. Pasteur*, xix. 9.—Musgrave (1904). Amebas, their cultivation and etiologic significance. *Bureau of Government Laboratories*, Biol. No. 18.—Musgrave and Clegg (1906, 1907). The cultivation and pathogenesis of Amoebae. *Philippine Journ. of Scicnce*, i.—Nägler (1909). Entwicklungsgeschichtliche Studien über Amoeben. *Arch. f. Protistenk.* xv. 53.—Noc (1909). Recherches sur la dysenterie amibienne en Cochinchine. *Ann. Inst. Pasteur*, xxiii. 177.—Schaudinn (1903). Untersuchungen über die Fortpflanzung einiger Rhizopoden. *Arbeit. a. d. Kais. Gesundheitsamte*, xix. No. 3.—Strong (1907), in Osler and McCrae's *System of Medicine*, i. 489.—Tanaka (1910). *München. med. Wochenschr.* No. 44, p. 2300.—Vahlkampf. Beiträge zur Biologie u. Entwicklungsgeschichte von *Amoeba limax*. *Arch. f. Protistenkunde*, v.—Viereck (1907). Studien über die in den Tropen erworbene Dysenterie. *Beihefte z. Archiv f. Schiffs- u. Trop.-Hygiene*, xi. Beiheft i. 7.—Walker (1908). The parasitic amoebae of the intestinal tract of man and other animals. *Journ. Med. Research*, xvii. 379.—v. Wasielewski and Hirschfeld. *Hyg. Rundschau*, No. 16.—Werner (1908). Studien über pathogene Amöben. *Beihefte z. Arch. f. Schiffs- u. Tropen-Hygiene*, xii. Whitmore (1911). *Centralbl. f. Bact. etc.* 1 Abt. Orig. lviii. Heft 3.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EXPLANATION OF PLATE VI.

N = Nucleus. C. V. = Contractile Vacuole.

Figs. 1–19 represent the amoeba of type (*a*).

Fig. 1.	Cysts.
Figs. 2, 3 and 13.	Escape of a single motile amoeba from the cyst.
Fig. 4.	Empty cyst shell.
Figs. 5 and 14.	Free motile amoebae.
Figs. 6–8 and 16.	Fission.
Figs. 9–11.	Formation of the "lobulate body."
Figs. 12 and 19.	Commencing encystment.
Fig. 17.	Amoeba containing endogenous buds.
Fig. 18.	Budding forms.

Figs. 20–28 represent the amoeba of type (*b*).

Figs. 20 and 24.	Cysts.
Figs. 21 and 25.	Free motile amoebae.
Figs. 22, 23 and 27.	Fission.
Fig. 26.	Group of amoebae containing endogenous buds.
Fig. 28.	Vacuolated amoeba with irregular chromatin masses.

Figs. 1–12 and 20–23 are drawn from unstained specimens.

Figs. 13, 15, 17, 18, 19 and 25 are drawn from specimens fixed with corrosive-acetic-alcohol and stained with iron-haematoxylin and eosin.

Figs. 14, 16, 24, 26, 27 and 28 are drawn from specimens fixed with osmic acid and stained by Giemsa's method.

The drawings were made with a Zeiss's drawing apparatus, compensating ocular No. 6; apochromatic 2 mm., aperture 130 immersion-lens, tube length 160 mm. Each division of the scale represents 2 μ.

A MICROFILARIA (*MICROFILARIA ROSENAUI* N. SP. FROM THE CALIFORNIA GROUND SQUIRREL (*CITELLUS BEECHEYI*).

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With Plate VII.

The following is a brief description of a blood worm which was first observed by Mr J. W. Kehoe, one of the technical assistants at the Federal Laboratory. The parasite has been found in squirrels coming from almost all parts of California east of the Sierra Nevada Mountains, and between the Mexican border and the Sacramento River (between 33° and 38° North Latitude).

Rodents from the southern sections of the State seem to show a higher percentage of infestation than those from further north. It is probable that at least five per cent. of the squirrels from the part of the State in which the parasites are found harbour the worm. There is no evidence that the nematode exercises any deleterious influence on the host. The adult forms of the parasite have not been found though careful search for them has been made.

Description of the worm: In the fresh blood attention is attracted to the parasite by a movement among the cells, and careful inspection shows this to be due to a very active worm. While the movements are quite vigorous no definite progress is made. The worm has been found alive two or three days after the death of the host. There is no evidence of the presence of a sheath. The tail is gently tapering while the head is rather blunt. The structure of the parasite cannot be studied satisfactorily in fresh preparations. The worm is readily stained by the ordinary basic dyes, but, for the study of the structure, Giemsa's method has given the best results. There is a faintly staining cuticle

L. H. Wilder. del.

which shows very fine transverse striations, but careful examination has failed to reveal any indication of a sheath. Within the cuticle is a column of nuclei which is more or less completely interrupted by several "clear spaces."

The nuclei: These are of two varieties: (1) nuclei usually spherical and staining uniformly deeply; these are by far the more numerous; (2) nuclei that are a trifle larger, somewhat less regular in outline and staining less deeply. The fourth nucleus from the tip of the tail is very generally one of the latter class. Others are scattered irregularly throughout the worm, except in the head and in the other clear areas.

Clear spaces: The head of the worm is always free from nuclei. About one-fourth to one-sixth of the distance from the head toward the tail is an area in which nuclei are almost invariably absent. About two-thirds of the distance from the head to the tail is a space in which cells are few in number. Near the tail, from one-eighth to one-fourth of the distance to the head, there is nearly always an area in which cells are absent. Some specimens show other clear spaces, but the ones mentioned are practically constant.

Dots or Granules: Almost invariably the clear space lying about two-thirds of the distance from the head to the tail will be found to contain a large number of fine granules. There are usually a few of these bodies, three or four or more, near the extreme front of the head, and a small number are often found in the tail. Occasionally granules are found in other parts of the worm, but usually they are confined to the head and to the clear space, two-thirds of the distance from the head to the tail. It was thought at first that these little objects, which are only seen in specimens stained with Giemsa stain, were artifacts, but their constancy leads to the conclusion that they are part of the structure of the worm.

Head: The head, which is cylindrical, tapers sharply to a flattened anterior extremity. The portion devoid of nuclei is usually about one and one-half times as long as the diameter of the worm.

Tail: The tail, which makes up about one-fourth of the length of the worm, tapers gently to a rather sharp point. Terminal nuclei in the tail are usually oval, the long axis corresponding to the long axis of the worm.

Measurements: The average length of ten specimens was 0·22 mm. (extremes, 0·17 mm. and 0·25 mm.). The width is from 0·0055 mm. to 0·007 mm., averaging a little less than that of the red blood corpuscles of the host.

DESCRIPTION OF A *HERPETOMONAS* PARASITIC IN THE ALIMENTARY TRACT OF THE COMMON GREEN-BOTTLE FLY, *LUCILIA* SP.

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(*From the Quick Laboratory, Cambridge.*)

With Plates VIII and IX and 2 Text-figures.

A *Herpetomonas* has been previously recorded in species of *Lucilia* by Patton in India (1908) and by Roubaud in the Congo (1908), but neither observer has described the parasite.

The following paper is concerned with the description of a *Herpetomonas* found in two species of *Lucilia* which infest butcher's shops in Cambridge. It is set out under the following heads:

Note on the digestive tract of Lucilia.

The alimentary tract of *Lucilia* is similar to that of *Musca domestica* and allied Diptera. The oesophagus (Fig. 1) leads to the midgut, which commences at a valve-like organ, the proventriculus. Before reaching the proventriculus, however, it branches to form the oesophageal

diverticulum, which is at first tubular, but later ends in a large bilobed sac, sometimes, though incorrectly, referred to as the 'crop.' The midgut is dilated slightly, and is succeeded by the small intestine, which terminates at the point of entry of the Malpighian tubules. The hindgut commences at this point, and ends in a dilated portion termed the rectum, which opens to the exterior at the anus.

In this paper the term 'crop' will be retained only for the sake of brevity.

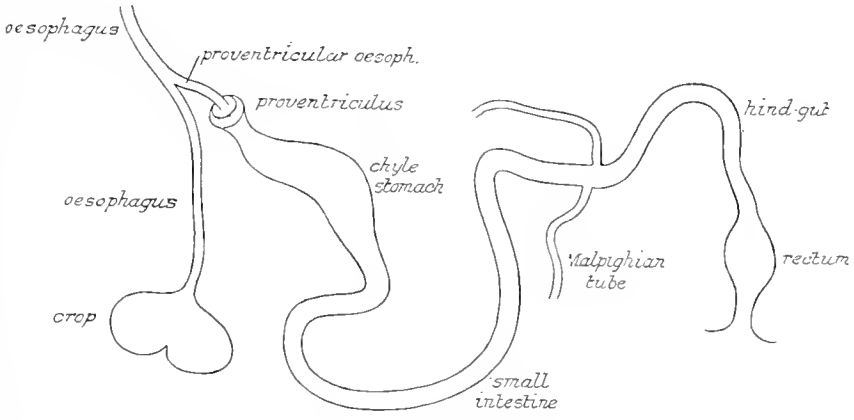

Fig. 1. Showing the anatomy of the alimentary tract of *Lucilia*.

The methods of examination of the gut contents.

The parasites were studied both in the living state and in stained preparations. Living specimens were obtained by dissecting out the flies' intestinal tract in saline solution, removing portions of it to a slide, and protecting with a cover-glass. Stained preparations were made from isolated portions of the gut, opened up in a small drop of salt solution and smeared out in the usual way. They were fixed in absolute alcohol, and finally stained with Giemsa diluted 1 : 20. It was found that a weak solution of the stain gave less diffuse staining than a stronger one.

Morphology.

The general character of the parasite varies in the different regions of the intestine in which it is encountered. The description of the various forms may therefore be conveniently arranged according as they are found (i) in the oesophageal diverticulum or 'crop,' (ii) the midgut,

(iii) the hindgut and rectum, although intermediate forms between the predominant types occurring in each region are occasionally found. The scarcity of such intermediate forms probably depends upon rapid and marked changes of form occurring in the parasites as they pass from one region of the intestine to another.

I. The parasite in the oesophageal diverticulum.

In this region, we find (1) *small truncate pyriform bodies* (the so-called cysts), (2) *oval forms without a flagellum*, and (3) *oval forms with a short flagellum*. These forms are connected by intermediate stages, and none of them show any signs of division. They have the following characters:

(1) The cysts, as seen alive, are small truncate pyriform bodies about $3{\cdot}5 \times 1{\cdot}5\ \mu$ in size. Their truncated appearance is due to a small cup-shaped depression in the protoplasm, at what we consider to be the anterior extremity of the cell. The protoplasm can readily be seen to consist of an inner core of endoplasm, invested by a layer of ectoplasm, which however does not cover in the endoplasm at the site of the above-mentioned depression at the anterior end of the cell.

In stained preparations, the ectoplasm, or 'periplast,' appears as a dense blue-staining coarsely sponge-like structure, while the endoplasm is more homogeneous, and appears pale-blue through the periplast, but in cells which have ruptured it can be seen to be stained pale-pink. As was seen in the living cell, the periplast does not completely cover in the endoplasm, but leaves a tiny area where the latter comes to the surface at the truncated end of the cell. The pale-staining endoplasm may be called the *cytopharynx*, and its cup-shaped entrance the *cytostome*. The *lip* of the cytostome consists of periplast. In those cysts which have the most differentiated protoplasm, the lip of the cytostome is inverted, probably by the action of the retracting endoplasm, so that only a minute aperture, or *micropyle*, is left opening into the cytostome. The periplast is thus made to fold over the exposed endoplasm. Embedded in the ectoplasm are the nucleus, blepharoplast, and 'chromidia'; and in the endoplasm traces of a flagellar apparatus can sometimes be seen as a faintly-staining red line. (See Pl. VIII, figs. 1–2, 23–25.)

The *nucleus* is round and placed eccentrically against the side of the cell. It consists of a delicately reticular matrix, which stains

pale-pink, and it is devoid of chromatin. It causes a slight projection of the periplast into the cytopharynx.

The *blepharoplast* is a small oval body constantly situated in the periplast at the posterior end of the cytopharynx, and when any traces of a flagellar apparatus are present in the cytopharynx, they are seen to run up towards it. In structure, the blepharoplast consists of a homogeneous-looking matrix, which stains rosy-red, and is devoid of chromatin. It can be distinguished from the chromidia, by which it is often surrounded, only by its staining reaction, the chromidia appearing deep carmine.

The *chromidia* consist of a group of deeply-staining carmine-coloured granules, each of which is often as large as the blepharoplast. The group has a characteristic position at the extreme posterior end of the cell, but very often an isolated granule occurs close to the blepharoplast, the significance of which is not obvious. The chromidia are often obscured from view when the periplast is densely stained. (Pl. VIII, figs. 23–25.)

(2) The *oval forms* are connected with the cysts by intermediate forms which are about the same length as the cysts, but broader. The typical oval forms are longer and possess a pointed anterior extremity, corresponding in position to the cytostome of the cyst. In stained specimens the protoplasm is seen now to be undifferentiated into the periplast and cytopharynx. The chromidia are scattered throughout the cell, and the nucleus and blepharoplast are partly chromatinised. It is consequently very difficult to distinguish the blepharoplast from one of the chromidia, but it lies to the side, or in front, of the centrally placed nucleus. (Pl. VIII, figs. 1–2.)

(3) *The oval forms with a flagellum* differ from the preceding forms only in possessing a free flagellum, the length of which equals that of the body of the cell, and in the nucleus and blepharoplast being more chromatinised.

The flagellar apparatus consists of (i) a rigid rod-shaped and heavily chromatinised body, the rhizoplast, lying between the blepharoplast and the anterior end of the cell, but having no chromatin connection with the blepharoplast; and (ii) the young flagellum, which consists of a delicate prolongation of the cell-protoplasm, staining pale-pink, investing a filament of chromatin, staining deep carmine, which runs into the anterior end of the rhizoplast (Pl. IX, fig. 6). In those cells possessing a divided rhizoplast, two chromatin filaments may be present in the protoplasmic prolongation of the cell, each arising from one of the

halves of the rhizoplast (Pl. VIII, fig. 5). Usually the blepharoplast shows no signs of division, but forms may occur in which it has divided, although there are no signs of division elsewhere.

Other forms may be seen with two chromatin filaments in the flagellum, as described above, but with no signs of division in the rhizoplast. (Pl. VIII, figs. 3–6.)

II. The parasite in the midgut and small intestine.

The parasites found in this region of the gut are quite distinct from those seen in the 'crop,' and intermediate forms are difficult to find.

Both in living and stained specimens, they are seen to be in a state of very active division, so that a portion of the gut sometimes contains literally myriads of them.

In the living state, the form of the parasite is seen to be very variable, but often it is like a greatly elongate cone with the flagellum at the base of the cone. The flagellum is long and wavy. The body of the cell measures 15 to 20 $\mu \times 3\,\mu$; the flagellum is 25 to 40 μ long. Dividing cells are broader.

The following structural details are observable in stained specimens:

(*a*) The cell protoplasm is finely alveolar and stains bright-blue, there being no differentiation into periplast and endoplasm. It contains the nucleus, blepharoplast, rhizoplast, and chromidia; and forms a fine membrane about the chromatin of the flagellum. In dividing cells the protoplasm has a coarser structure.

(*b*) The nucleus is spherical and situated in the centre of the cell. It consists of a reticulate achromatinic matrix, staining pale-pink, in which lies a network of chromatin, thickened at the nodes of the reticulum. The following stages in the structure of the dividing nucleus may be observed:

(i) The nucleus consists of the achromatinic matrix without the finer parts of the chromatin network, so that the latter appears coarser and the nodal points thicker. (Fig. 2 *b*.)

(ii) The nucleus has an oval form, and lies across the cell; the chromatin granules are aggregated around the periphery of the nucleus, and also in a transverse line across its longest diameter. (Fig. 2 *d*.)

(iii) The nucleus shows chromatin granules which have become flattened out, forming a peripheral ring of chromatin, and a transverse band lying nearly across the nucleus, but not touching the peripheral ring. (Fig. 2 *f*.

(iv) In this stage the transverse band of chromatin is divided into two equal parts. (Fig. 2 *g*.)

(v) The nucleus is seen to have divided into two spherical daughter nuclei, each with a peripheral ring of chromatin and a short transverse band of chromatin. (Fig. 2 *h*.)

(vi) The further stages are represented by nuclei in which the chromatin network is reformed by fragmentation of the chromatin bands and the dissemination of the fragments to form a fine network in the nucleus. (Fig. 2 *i*, *k*, *l*.)

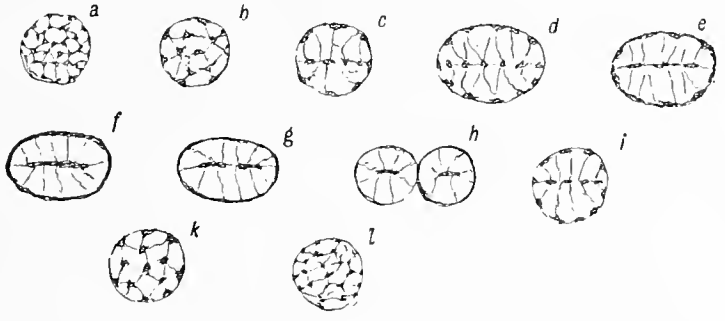

Fig. 2. Showing the structure of the dividing nucleus of *Herpetomonas luciliae*.

(*c*) The blepharoplast lies midway between the nucleus and the anterior end of the cell, and is of very variable form (Pl. IX, figs. 1–14). In the 'resting' cell it is apparently a homogeneous mass of chromatin; but in dividing cells, the chromatin splits into two halves, which separate and reveal a lighter-staining intervening matrix.

(*d*) The rhizoplast is in the same position as in the 'crop'-forms, but it is thicker and contains more chromatin, and therefore stains very densely. In some forms the rhizoplast is split longitudinally, and in such cases it may be connected with either one or two chromatin filaments in the flagellum.

(*e*) The flagellum, in 'resting' cells, consists of a chromatin filament surrounded by an extension of the cytoplasm, as in the 'crop'-forms. In some cases, whether the rhizoplast be divided or not, the flagellum contains a second filament of chromatin which has no connection with the first filament. The two filaments, which may be very different in length, are connected together by a pink staining cytoplasmic membrane. In some of the forms containing two chromatin filaments the membrane stretched between the filaments is split either proximally, or distally, or both; and in other cells the division is complete, the cell then possessing two independent flagella. In such

cells none of the organella may be divided, a unicellular biflagellate creature being the result, but these forms are very rare. In yet other cells we find a cell, incompletely divided, with *four* chromatin filaments in the flagellum, which must be interpreted as cells in which redivision is taking place prior to the separation of the daughter cells.

(*f*) The chromidia are scattered throughout the cell. Relatively to the other structures they are very much smaller than in the 'crop'-forms. The more actively dividing parasites seem to contain the most granules.

The structure of the parasites in this region is therefore very variable, in that they do not form a gradual series of forms. This indicates that the sequence of division of the various organella is not uniform; for instance in some cells only the flagellum has divided, in others only the rhizoplast. (Pl. IX, figs. 1–14.)

III. The parasite in the hindgut and rectum.

In this region the forms of the parasite fall into three main groups, which are connected by intermediate forms: (i) large oval flagellates, (ii) round flagellates, (iii) small oval forms, or 'cysts.' None of these show any signs of multiplication, either in living or stained preparations.

(i) The *large oval* forms are found in the hindgut immediately below the opening of the Malpighian tubes. They are less numerous than the other types, probably because this stage is very transitory. They measure 8 to $10\,\mu \times 2$ to $3\,\mu$. The cytoplasm, as in the midgut forms, is not differentiated into periplast and cytopharynx, and stains pale-blue. The nucleus, blepharoplast, and flagellar apparatus contain less chromatin than the corresponding structures in the midgut forms, and the matrix of the nucleus, which stains pale-pink, is more evident. The blepharoplast still occupies the same relative position to the nucleus as in the midgut forms, that is, well in the front end of the cell. The flagellum is much shorter than in the midgut forms, and stains less deeply owing to its being partly dechromatinised. The chromidia have a tendency to become grouped in the posterior end of the cell. (Pl. IX, fig. 7.)

(ii) The *round* forms are chiefly found in the posterior part of the hindgut, and in the rectum. In this category we include forms which are not round but oval, for the only difference between them is their shape. They are of the same length, about $5\,\mu$, but whereas the round cells are about $5\,\mu$ in breadth, the oval cells have contracted to about

2 μ. The latter are very abundant indeed in the rectum. It appears that the large oval forms become rounded up, and that they then contract from side to side.

The cytoplasm is differentiated into ectoplasm and endoplasm: in forms probably representing a later stage of development, the differentiation is extreme, a very dense-staining periplast being formed. The appearance thus presented is the same as that of the forms occurring in the 'crop,' which have been described above as cysts. The nucleus, blepharoplast, and flagellar apparatus have even less chromatin than in the large oval forms. The nucleus, blepharoplast and intracellular part of the flagellar apparatus may even lose all their chromatin; the nucleus then staining pale-pink, the blepharoplast rosy-red, and the intracellular flagellum being invisible, probably because the dense periplast obscures such a delicate structure.

The blepharoplast is now situated to one side of, or even just behind, the nucleus which is slightly eccentric. The rhizoplast as long as it contains chromatin is seen to follow the blepharoplast as this passes backward, although there is no visible connection between them.

The extracellular part of the flagellum is faintly stained but is seen often when the intracellular part is invisible. The chromidia are very large in those cells in which the organella have lost most chromatin: they are often with difficulty distinguished when the periplast is densely stained. A single chromidium away from the others is sometimes seen close to the blepharoplast. The others are grouped at the posterior end of the cell in the periplast. (Pl. VIII, figs. 10–22.)

(iii) The *cysts* are found in the lower end of the rectum in very great numbers, but are always discrete. They have been described above under the 'crop'-forms. (Pl. VIII, figs. 23–25.)

Taxonomy.

The parasite above described must be classed with those described by Prowazek, Patton, Mackinnon, and others, and named *Herpetomonas*.

It has been recently suggested by Miss Mackinnon (1910) that there has been needless subdivision of this genus into species without consideration of the adaptability of perhaps one species to various hosts. I had this in mind at the time (1909) of conducting the observations on this parasite, and accordingly dissected some *Musca domestica*, and *Calliphora coerulea*, from the same source, two butcher's shops, as the *Lucilia*, but was unable to find herpetomonads in them.

This species is therefore probably distinct from *H. muscae domesticae* at least, and I therefore propose for it the name *Herpetomonas luciliae.*

It has been said that such parasites as these are 'biflagellate,' because certain forms appear to have two flagella. The appearance, however, is deceptive, and these parasites in reality only have one flagellum in which the two chromatin filaments stain very prominently. The term 'biflagellate' is only applicable to those forms, rare in *H. luciliae,* in which there are two *free* flagella.

Prowazek, Chatton, and others would use this inconstant form of the parasite—the so-called biflagellate form—with which to define the genus *Herpetomonas,* and would separate it from *Leptomonas,* which has a slightly different type of flagellar apparatus.

Miss Mackinnon, however, has shown that a 'Leptomonas' in the larval host may become a 'Herpetomonas' in the imaginal host, and that the difference in the flagellar apparatus is only a matter of the time at which the flagellum divides. It seems therefore wrong to class these parasites into two genera.

They would thus all be named *Herpetomonas,* sensû Pattoni, who defines them as having no undulating membrane and the blepharoplast near the anterior end of the cell.

The method of infection of the fly.

The experiments performed in order to discover how the parasite is transmitted from fly to fly were conducted in the following manner.

An attempt was made to raise 'clean' flies in the laboratory, but owing to the cold summer (1909), and also because the experiment was begun rather late in the year, the pupae obtained did not hatch out. Recourse was therefore had to flies caught wild, and in estimating the results obtained with these it was borne in mind that 76 % of them had been previously found infected.

The flies were kept in a large fly net, but they were with difficulty kept alive. They were fed on fresh meat contaminated with the rectal parasites of other flies, and afterwards were killed at definite intervals and examined for parasites.

The result was meagre, but three flies which had been fed as described above showed actively growing cysts in the 'crop' two days later; whereas of flies examined straight from nature, I never found one containing these forms in the 'crop.'

The fact that rectal cysts are found in the 'crop' of the fly indicates that the parasite is transmitted from fly to fly by contamination of its pabulum.

Hereditary transmission does not take place. Several larvae from eggs laid by infected flies were dissected, but parasites were never found. Neither do these larvae become infected if they feed on contaminated meat, this being in contrast to Miss Mackinnon's observations (1910) on the life history of *Herpetomonas* from dung-flies.

In the case of *H. luciliae*, therefore, I conclude that infection is usually produced in the imago by its feeding on pabulum that has been contaminated with cysts, derived from other infected flies.

Biology.

Movement. The parasites that occur in the 'crop' and hindgut, each possessing only a short flagellum, have little power of progression. The flagellum waves feebly to-and-fro, but without causing much movement. Those with a long flagellum, as in the midgut, can only with difficulty be kept within the field of vision, the flagellum being generally directed forward under these circumstances. The 'cysts' are quite inert, floating about in the fluid in which they are examined.

Growth and division. At various stages in the life-history of the parasite, the relative rapidity of its growth and its change of structure can be estimated by noting the comparative numbers of the parasite at that stage. In the 'crop' the cysts and the oval forms without a flagellum are the rarest, so it must be assumed that these forms are the most rapid in their growth. In the midgut the parasite does not remain long without division; while in the hindgut and rectum the cysts and the small oval forms with a flagellum are found in enormous numbers, and consequently represent the slowest stage of growth.

It seems therefore that rapidity of growth and change of structure is most rapid in the upper part of each subdivision of the gut, while at the lower end it is slowest and there is not much change of structure in the parasite. Division only occurs while the parasite is in the midgut.

Individual variability. The study of stained specimens of the parasite shows that it is very variable in structure, particularly in the forms in the midgut, which we may regard as at the most plastic stage.

Physiology.

Cytoplasm. The cytoplasm of the midgut forms shows no differentiation in structure, and therefore its function is probably generalised; but in the forms occurring in the 'crop' and hindgut, it is more or less differentiated into ecto- and endoplasm. The ectoplasm forms the periplast, the function of which is doubtless to resist desiccation of the cell. It is accordingly most marked in the cysts which, when voided in the excreta, have to withstand the effects of atmospheric drying, perhaps for long periods. From its structure it seems to be formed out of the more solid spongy elements of the cytoplasm. When water and food are plentiful, as in the 'crop,' the periplast soon disappears, which proves that this structure is ill-adapted for the absorption of water and nutritive substances. On the contrary, in the hindgut, where the excreta of the fly are more and more concentrated, the periplast is rapidly formed.

The reduced metabolism of the cell is then carried on by the endoplasm, acting through the small area which comes to the surface of the cell at the 'cytostome.' The endoplasm is thus well-named the cytopharynx. It seems, from its structure, that this endoplasm is derived from the more fluid 'hyaloplastic' part of the cytoplasm.

Later on, as shown by the movements of the chromidia, the ectoplasm and endoplasm diffuse into one another, and then the whole surface of the cell is available for absorption. The cytostome would be a weak spot in the armour of the cell under conditions of excessive dryness, were it not for the following changes. As more dryness is experienced the cell loses water, thereby causing the cytopharynx to retract, and consequently deepening the cytostomic pit. The effect of this is to invert the periplastic lips of the cytostome over the vulnerable area, with the exception of the tiny micropyle described above. The cell is thus enabled to withstand prolonged desiccation.

Rhizoplast. The function of the rhizoplast seems to be the secretion of the chromatin filament in the flagellum. This is probably effected by the protoplasmic achromatic basis of the rhizoplast. When the rhizoplast loses its chromatin this protoplasmic basis is possibly retracted into the blepharoplast, in view of the close connection which exists between the two structures.

Flagellum. The flagellum, which, it must be reiterated, consists of two distinct parts (i) the protoplasmic basis, and (ii) the chromatin

filament, is lost in the following manner. The chromatin filament first gradually disappears, but before this change is complete the rhizoplast has passed backward in the cytopharynx, drawing the flagellum after it. By this means the free part of the flagellum is shortened. It constitutes an *active* shortening of the flagellum. Prowazek has previously described this process in the flagellum of *H. muscae domesticae.* The chromatin filament in the flagellum is then absorbed centripetally, so that in some cells only the chromatin of the intracellular part remains, and, as this process takes place, the protoplasmic basis of the flagellum is retracted *pari passu.* The protoplasmic basis does not remain when the chromatin has been absorbed. This constitutes a *passive* shortening of the flagellum.

These observations on the structure and changes in the flagellum seem to show that the chromatin is devoted to sustaining the flagellum in a filamentous form, which the protoplasm surrounding it has no power *per se* of doing.

The function of movement is, we think, subserved by the protoplasmic basis of the flagellum. It is obvious that the protoplasmic basis is homologous to the undulating membrane of *Crithidia* and *Trypanosoma,* although it has been seen that when the flagellum is drawn up into the cell by the posterior movement of the blepharoplast it does not form an undulating membrane as such but remains within the endoplasm. The undulating membranes of the Trypanosomatidae contain myonemes, so that it is most probable that the function of movement is subserved by the protoplasmic basis of the flagellum. The staining reaction of the protoplasmic basis, or of the *kinetoplasm,* as I propose it be called, is the same as the endoplasm of the cell, and is obviously derived from it, so that it is interesting to see that the more hyaloplastic constituents of the cell are employed for the function of movement.

Chromidia. It has been seen how the size and number of the chromidia vary inversely with the amount of chromatin present in the nucleus and other organella. There can therefore be little doubt that they represent the supply of chromatin for the use of the cell. Swellengrebel has shown that they do not consist of chromatin itself, but of a slightly altered substance named 'volutin.' They probably are concerned in the following manner. When the parasites are undergoing involution the chromatin in the organella is dissolved by the protoplasm and circulates in the cell, and is then taken up by certain plastids, and resecreted as 'volutin,' in the form of the chromidia.

The reverse process takes place when the cell is on the 'up-grade,' the volutin being dissolved and resecreted by the protoplasmic basis of the nucleus, blepharoplast, and flagellar apparatus, respectively.

The process is familiar in certain plant-cells, such as *Spirogyra*, in which the carbohydrate metabolism is similar.

The presence of volutin has been shown by Nuttall (1910), and by Hindle (1910), to be associated also with degeneration of the cell, but in this *Herpetomonas* the most vigorous parasites are seen to contain the most volutin granules, or chromidia.

Summary and conclusions.

1. The parasite described inhabits the alimentary tract of two species of *Lucilia*. In addition it has a resting stage—the 'cyst'—which is passed in the voided excrement of the insect.

2. When the cyst is ingested by a fly it grows in length and becomes flagellated in the 'crop'; in the midgut it greatly elongates and multiplies rapidly by division; in the hindgut it first shortens, then becomes spherical, and finally oval and cyst-like, meanwhile losing its flagellum.

3. The cysts measure 3 μ, the fully flagellate forms 20 μ, in length.

4. The cysts possess a *cytostome* and *cytopharynx*, the functions of which are probably nutritive.

5. The flagellum consists of two parts (i) a cytoplasmic part, which probably subserves the power of movement and which I therefore propose to call the *kinetoplasm*, and (ii) a thread of chromatin secreted by the rhizoplast.

6. The function of the chromatin in the flagellum is probably to sustain a filamentous form.

7. The nucleus divides by a definite process something akin to mitosis.

8. The chromidia serve as reserve material for the supply of chromatin in the cell. They are secreted probably by cell-plastids.

9. I propose the name of this parasite be *Herpetomonas luciliae*, and that the genus *Herpetomonas* should be defined so as to include the forms described as *Leptomonas*.

REFERENCES.

Chatton, E. (1909). Sur un trypanosomide nouveau d'une Nycteribie, et sur les relations des formes *Trypanosoma* etc. *Comp. Rend. Soc. Biol.* lxvi. pp. 981, 982.

Hindle, E. (1910). Degeneration phenomena of *Trypanosoma gambiense*. *Parasitology*, iii. pp. 424–435.

Nuttall, G. H. F. (1910). The degenerative appearances observed in *Piroplasma canis* and *Trypanosoma brucei* etc. *Parasitology*, iii. pp. 203–210.

Patton, W. S. (1908). *Report of the King Institute*, Madras.

Prowazek, S. (1904). Die Entwicklung von *Herpetomonas* etc. *Arb. a. d. Kaiserl. Gesundh.* xx. pp. 450–452.

Roubaud, E. (1908). Sur un nouveau flagellé parasite etc. *Comp. Rend. Soc. Biol.* lxiv. pp. 1107, 1108.

EXPLANATION OF PLATES VIII—IX.

PLATE VIII.

Figs. 1–6. Forms found in the 'crop.'

Fig. 1. Oval form without flagellum. The chromidia are scattered throughout the cell. The blepharoplast is at the extreme posterior end. It can hardly be seen in the figure.

Fig. 2. Oval form without flagellum. Nucleus slightly chromatinised.

Figs. 3–6. Oval forms with flagellum.

Fig. 3. Shows the blepharoplast dividing, and a single flagellum.

Fig. 4. Shows the cell with the rhizoplast undivided and the flagellum with two chromatin filaments, between which is stretched the kinetoplasmic membrane.

Fig. 5. Shows the rhizoplast divided, as well as the 'double' flagellum.

Fig. 6. A densely staining form in which the nucleus cannot be seen.

Figs. 7–22. Forms found in the hindgut and rectum. In all the cytopharynx is well-marked, but the nuclei are badly marked in the figures.

Fig. 7. Large oval form with small chromidia.

Fig. 8. Shows the chromidia in a group at the posterior end of the cell.

Fig. 9. Shows the nucleus, blepharoplast and flagellum all more or less dechromatinised.

Figs. 10–14. Round forms.

Fig. 10. Shows commencement of the formation of the cytopharynx, the blepharoplast lateral to the nucleus.

Fig. 11. Shows well the blepharoplast at the posterior end of the cytopharynx. The nucleus shows one of the stages of mitosis.

Fig. 12. Partial retraction of the cytopharynx has occurred.

Fig. 13. The chromidia are grouped at the posterior end of the cell. The rhizoplast is still chromatinised.

Fig. 14. Shows the differentiation of the protoplasm into periplast and cytopharynx.

Figs. 15–22. Oval forms with a flagellum. They all show the further differentiation of the protoplasm, and the consequent formation of the truncate end of the cell. The flagellum is short in all cases and is nearly dechromatinised; it cannot be seen when

it becomes intracellular. The blepharoplast is always at the bottom of the cytopharynx and stains less deeply than the chromidia.

Figs. 23–25. Cysts found in the rectum or 'crop.' The cytostome and micropyle are well seen ; also the characteristic position of the nucleus, blepharoplast, and chromidia.

PLATE IX.

Figs. 1–14. Various forms of the flagellate found in the midgut.

Fig. 1. Small cell in a resting condition.

Fig. 2. Cell showing two free flagella, the rhizoplast slightly divided.

Fig. 3. Cell in which the blepharoplast is dividing, showing the achromatic matrix.

Fig. 4. Cell showing formation of a second chromatin filament, with a protoplasmic membrane (kinetoplasm) between the two filaments. Nucleus showing mitotic figure.

Fig. 5. Cell showing division of the blepharoplast and rhizoplast; also the formation of a second chromatin filament.

Fig. 6. Shows division in nucleus, blepharoplast and rhizoplast. Flagellum with one chromatin filament in a thick sheath of kinetoplasm.

Fig. 7. Cell in which the nucleus shows mitosis; the rhizoplast is divided and the flagellum contains two chromatin filaments.

Fig. 8. The blepharoplast is dividing and the flagellum contains two chromatin filaments.

Fig. 9. Cell showing complete division except between the two chromatin filaments.

Fig. 10. Shows clearly the kinetoplasmic membrane.

Fig. 11. Flagellum twisted on itself. Rhizoplast and blepharoplast dividing.

Fig. 12. Cell showing complete division of all the organella, but not of the cell protoplasm.

Fig. 13. Complete division of all parts except the flagellum which is only split partially, both proximally and distally, new chromatin filaments being laid down by the side of the old.

Fig. 14. Cell showing a further stage of division. The two cells have completely divided and the formation of the new chromatin filaments is continued. The respective rhizoplasts of the two daughter cells have divided.

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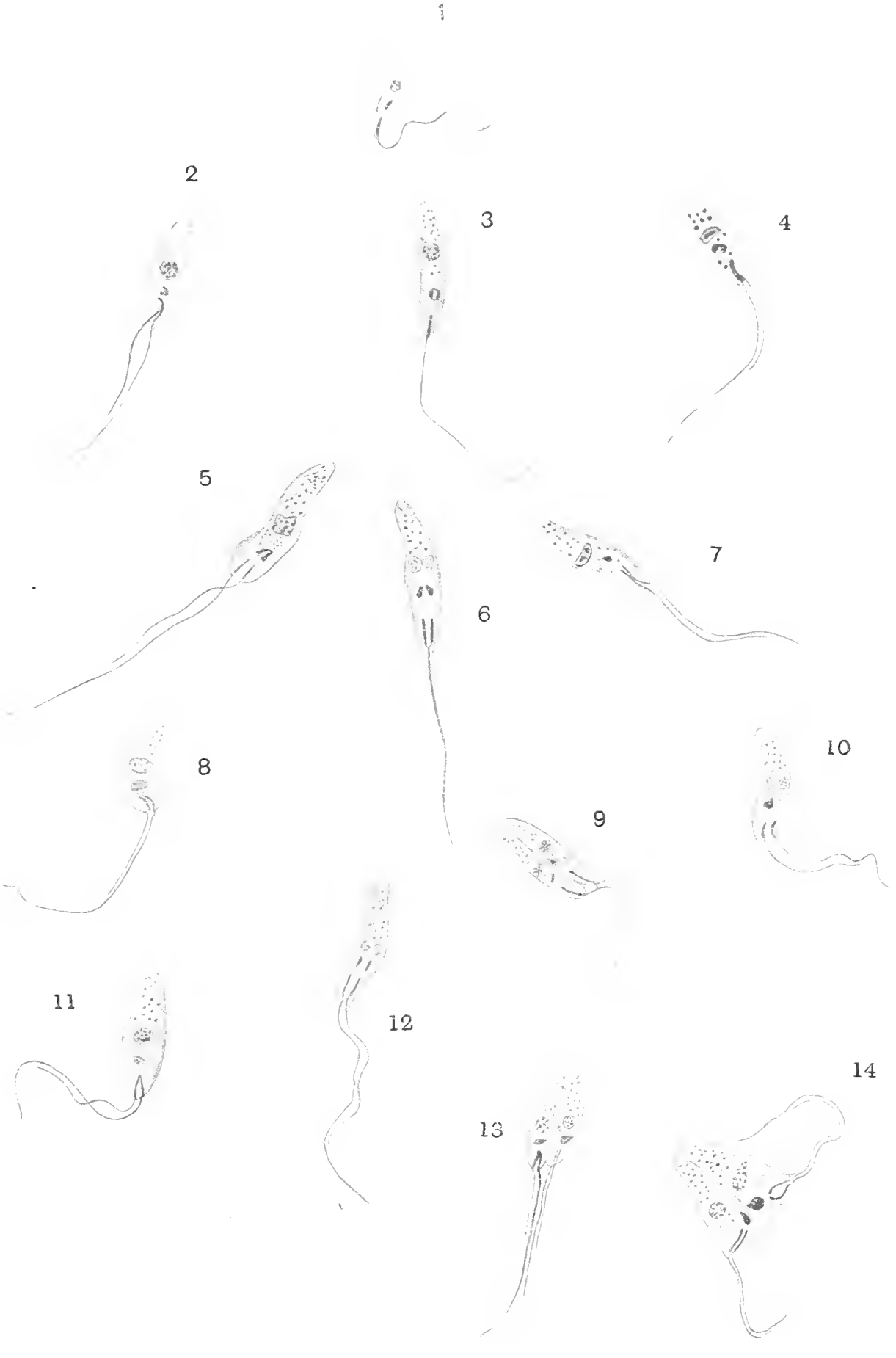
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THE STRUCTURE AND LIFE HISTORY OF *CRITHIDIA PULICIS*, N. SP., PARASITIC IN THE ALIMENTARY TRACT OF THE HUMAN FLEA, *PULEX IRRITANS*.

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With Plate X.

CONTENTS.

Introduction.

For some years now much of my attention has been given to the investigation of various Flagellates parasitic in Invertebrates. These parasites were various Crithidia and Herpetomonads and the hosts have been chiefly Hemiptera and Diptera. During the last three years, I have conducted research on the endoparasites of the fleas found on the

human body, mainly using *Pulex irritans*, the common human flea. Some of the *P. irritans* contained the parasite that I have named *Crithidia pulicis*, a typical *Crithidia*, with its life history complete within the flea and to this parasite the present paper refers.

Material and Methods.

The fleas used for the study of *C. pulicis* were specimens of *P. irritans* obtained from various parts of England. The work has involved much time and trouble, for I used only *bred* fleas for my research, and, at considerable personal inconvenience, bred and reared the fleas upon my person, keeping them in confined areas by means of celluloid and rubber "flea-cages" of my own designing. In order to avoid all possibility of contamination of the fleas by feeding on other animals, *e.g.* cats, dogs or rats, the fleas were bred to the third generation and these were the fleas used for dissection. No blood other than my own was used for feeding the fleas, a point on which I would lay stress. An interval of two days after the last feed was usually allowed to elapse before dissection as the preparations of *C. pulicis* were cleaner than if the fleas were dissected immediately after feeding.

The alimentary canals of about 200 adult and larval fleas were carefully dissected and preparations made of their contents. Much time was given to the examination of fresh preparations of the *Crithidia* contained therein. The percentage of infected fleas was low, and hence much waste of time occurred in the breeding of "unprofitable" hosts and in examination of the same. All other organs of the fleas were examined and faeces of the fleas were constantly searched.

With regard to stained preparations, both wet and dry methods of fixation were used. The chief stains used were iron-haematoxylin, Delafield's haematoxylin and modifications of the Romanowsky stain, especially Giemsa's stain. Methylene blue was useful for *intra vitam* staining. Fixation by osmic vapour followed by absolute alcohol proved quite satisfactory. Formalin vapour followed by absolute alcohol also was useful.

In connection with the occurrence of a *Crithidia* in the human flea bred in the manner described previously, I should like to state that the numerous detailed examinations made of my blood by smears, thick films and cultures have entirely failed to reveal the presence of any trypanosome. The bearing of this statement will be discussed later.

Distribution of Crithidia pulicis *in* Pulex irritans.

Crithidia pulicis shows three characteristic phases in its life history and is thus a typical *Crithidia*. It occurs throughout the alimentary tract of its host, both adult and larval fleas containing the parasite. The small oval or rounded pre-flagellates, having some resemblance to the Leishman-Donovan bodies, are most abundant in the entire alimentary tract of the larval fleas and in the crops of young adults. Fully developed flagellates, either growing or in process of division, are best observed in the midgut and intestine of the adult flea, while the post-flagellates are most numerous in the rectum of mature fleas and have often been recovered from the faeces of infected hosts.

Forms other than non-flagellates have not been found in the mouth or proboscidal regions of the flea, nor have fully developed flagellates been observed in the oesophagus of adult fleas.

Up to the present, there is no evidence of the occurrence of *C. pulicis* in organs other than those of the digestive tract. Hereditary infection, which has been fully demonstrated in *C. melophagia*, has not been shown so far in *C. pulicis*.

Movements.

C. pulicis is very active in its movements which are brought about chiefly by the undulating membrane, the flagellum being relatively short compared with that of such *Crithidia* as *C. gerridis*. *C. pulicis* moves with the anterior (flagellar) end forwardly directed and progression is aided by waves of contraction that pass from behind in a forward direction along the body. The wave-like motion is due to the myonemes in the body and membrane, which myonemes can be seen in life by the aid of the paraboloid condenser. The same phenomenon has been observed in living Spirochaetes and in *C. melophagia*. The myonemes in neither of these cases are artifacts, as some recent dogmatic writers would have us believe. Living organisms do not consistently display artifacts. The myonemes of the membrane are more marked than those of the body.

C. pulicis is capable of rapid reversal of its direction of motion. This is brought about by the organism swinging rapidly in a semi-circle, the posterior end acting as a centre of rotation.

Movements of flexion are more common in *C. pulicis* than in any other *Crithidia* that I have examined. The posterior end of the body

is very mobile and at times folds completely over so that the posterior end of the body lies parallel to the anterior end and the parasite is looped like a U. In ordinary quiet movement the body of the parasite may turn on itself, so that the posterior end seems folded just above the nucleus. (Pl. X, fig. 13.)

Entanglement movements were fairly common, but aggregation rosettes of *C. pulicis* were not nearly so numerous as in the case of *C. melophagia* or *C. gerridis*. One point about the aggregation rosettes of *C. pulicis* is the remarkable billowy effect due to the undulations of the membranes of the parasites.

Movements occurring during longitudinal division will be described in the section dealing with division.

Morphology.

Crithidia pulicis, like other insect flagellates, exhibits the three typical phases of a *Crithidia* in its existence. While the general life history is quite typical, there are many points that apply to *C. pulicis* only, and hence I prefer to establish a new species for it, rather than amalgamate it with *C. ctenophthalmi*, the parasite of the rat flea, *Ctenophthalmus agyrtes*.

The Pre-flagellate Stage.

Pre-flagellates (Pl. X, figs. 1–8) of *C. pulicis* are most abundant in the whole length of the alimentary canal of the larva of *P. irritans* or in the crops of young adults. They are oval or rounded bodies in their simplest form, measuring 2·3 μ to 7·0 μ by 1·5 μ to 4·5 μ. The general cytoplasm is faintly granular (figs. 1–8). The nucleus is well marked (figs. 1, 5, 6), and contains more chromatin than that of most other *Crithidia*. A small karyosome may be present (figs. 1, 6). The shape of the nucleus has a general resemblance to that of the entire pre-flagellate. The blepharoplast is large (figs. 4, 7, 8) and is either straight (figs. 2, 8), oval (fig. 7), or slightly curved (fig. 1). It is very obvious in life. In the older pre-flagellate (figs. 6–8), a clear area can often be distinguished in life, either to one side of the nucleus or below the blepharoplast, its position depending on the amount of elongation that the parasite has undergone. This is the area from which the flagellum develops. It takes the chromatin colouration in stained preparations. When the pre-flagellate elongates, the flagellar

end is the more rapid in growth, the endoplasm flowing forwards and pushing the ectoplasm before it. The contents of the chromatophile area concentrate and gradually differentiate into a thread (fig. 4), which gains the surface and forces the ectoplasm outwards forming the wavy membrane. The final portion of the thread protrudes beyond the body as the free flagellum (figs. 6–8). The posterior end of the flagellate develops less rapidly than the anterior end. Division may occur while the pre-flagellate stage is merging into that of the flagellate, and repeated division produces rosettes (figs. 3, 5, 8). All the pre-flagellates do not develop at the same rate, consequently some of the members of a rosette are still oval while others have already developed their flagella and membranes to some extent (fig. 8).

The somewhat frail appearance of the pre-flagellates is certainly in contrast with the relatively gross facies of the mature flagellate, but the whole development has been watched repeatedly in life and is unmistakable.

The Flagellate Stage.

The flagellate form of *C. pulicis* (figs. 9–17) is an organism measuring from 26 μ to 65 μ long, the free flagellum being included. The body is elongate and the flagellar end prolonged and finely tapering. The aflagellar posterior end is relatively blunt, being somewhat rounded in the fully formed individual (figs. 16, 17). The general protoplasm is richly granular but the granules are fine. The nucleus is oval, rich in chromatin, the chromatin being in the form of refractile granules in life and staining well whatever stain be employed (figs. 10, 12, 17). Its position in the body varies somewhat. Frequently it is about a third of the way between the two ends of the organism (figs. 14, 16, 17), but occasionally after periods of great activity of the parasite, it is found nearer the flagellar end.

The blepharoplast is large, usually lying horizontally across the body of the organism (figs. 9, 11, 15), and as a rule extending almost across the transverse diameter. Commonly it presents a homogeneous structure except when in division. Its shape may be oval (figs. 10, 15–17), rod-like (figs. 11, 13), or curved slightly. All the forms have been seen in life and the stained specimens merely corroborate the observations *in vivo*. In a few cases, a vertical blepharoplast has been found. This is uncommon but it has been recorded in many other *Crithidia*. There are a few chromidia (fig. 16) present in many of the

flagellates, and there seems to be a tendency for their number to increase just prior to division. As at this time the organism is passing through a critical period, the migration outwards of chromidia may be an adaptation to secure a more perfect equilibrium between the general cytoplasm and the nuclear elements.

One of the most noticeable features about *C. pulicis* is the beauty of its membrane. The undulating membrane is large and well marked. Its chromatic border is highly refractile in life, and stained specimens show it as a well-marked chromatin containing edge to the membrane (figs. 15–17), continued outwards just beyond the body as the free flagellum. Contractile myonemes (figs. 13, 15, 16, 17) are present in the membrane and faint body myonemes (figs. 9, 16) are also seen occasionally. The free flagellum and the chromatic border of the membrane show a transverse striation in a few cases. There is much morphological variation among the full-grown flagellates, depending on the frequency of and variation in division.

The Post-flagellate Stage.

The post-flagellates of *C. pulicis* (figs. 23–36) again show differences from most other *Crithidia*. In the majority of these organisms division into four without subsequent growth seems to be a common procedure prior to the formation of post-flagellates. In *C. pulicis* division into two, followed by some slight amount of growth seems general. But as flagellates of different sizes may divide, the range of size of the cysts is fairly great. The fully formed post-flagellate is from $3\,\mu$ to $6\,\mu$ long and from $2{\cdot}0\,\mu$ to $4{\cdot}6\,\mu$ broad in those that I have examined. The processes leading up to the full post-flagellate stage may be herewith summarised. Retraction of protoplasm from both ends of the organism towards the nucleus occurs (figs. 23–25); part of the protoplasm is absorbed. The chromatin constituent of the membrane appears to be absorbed and the free flagellum shortens (figs. 23–28). General fusion of membrane and body occurs and the organism becomes ovoid (figs. 29–36). A thin, gelatinous secretion appears on the outside of the ovoid mass and hardens into a varnish-like cyst wall around the contents. The fully formed post-flagellate (figs. 29–36) consists then of a thin coat enclosing the nucleus and blepharoplast together with a fair amount of protoplasm. The nucleus is often round but it may be oval. As a rule, the nucleus and blepharoplast remain quite distinct and slightly separated one from

another (figs. 28, 33, 35, 36) but in a few cases the nucleus and blepharoplast were apposed (figs. 30, 31, 34). On rare occasions the contents were slightly contracted from the varnish-like wall (figs. 34–36).

Division.

(*a*) *The Pre-flagellate.*

After the introduction of the parasite into a new host by way of the mouth, division occurs. As a result of repeated binary fission followed by growth, rosettes of pre-flagellates (figs. 3, 5, 8) are fairly common. The sequence of events in nuclear division resembles that of the flagellate stage described below. The chromatophile area of the pre-flagellate, however, divides, generally symmetrically, and hence each new pre-flagellate is a replica of its parent.

(*b*) *Division of the Flagellate.*

Longitudinal division of *C. pulicis* (figs. 18–22) is of two types:— (1) equal or symmetrical division; (2) unequal or asymmetric fission. The processes have been observed in life and corroborated by reference to stained specimens. Division is initiated by the concentration of the substance of the blepharoplast into two masses, one at either end (fig. 18). The blepharoplast becomes bowed and presents a dumb-bell appearance (fig. 19). The two heads of the dumb-bell finally become separated (figs. 20, 21). Soon after division of the blepharoplast has commenced, that of the nucleus begins, but the process is less rapid than is that of the blepharoplast. The flagellum and membrane next commence to split and as the flagellum violently lashes outwards, the division of the body follows. The two bodies thus formed gradually diverge (fig. 21) and a V-shaped organism then is seen. Ultimately the diverging arms of the V lie in a straight line (fig. 22) and the two daughter forms finally separate by constriction at the posterior end.

In asymmetric division two types are encountered:

(1) The blepharoplast and nucleus divide into unequal portions and the body fission follows that of the chromatic masses (fig. 22).

(2) On rare occasions the nucleus and blepharoplast may divide symmetrically while the general protoplasm divides asymmetrically.

Division is a rapid process and there is a great variety of forms resulting therefrom. I have not found division rosettes of the flagellate form of *C. pulicis* at all common. After the primary division, a second

may rapidly follow, but the original dividing organisms have separated from one another as a rule, so that rosettes are not produced.

Division of the flagellate form occurs just previous to the formation of the post-flagellates, but the number of divisions appears to be less than in the other *Crithidia* I have studied, and as a result, the ovoid post-flagellates of *C. pulicis* are relatively larger.

The form of the parasite is influenced to some extent by the diet of the host. In a starved flea the body cytoplasm of the flagellates is very finely granular. A short time after fresh blood reaches these parasites, they become larger, more richly granular and division commences. Flagellates occurring in the semi-digested "dark" blood in the hinder part of the alimentary canal of the flea are larger on the average than those in the fore part, the food medium apparently being more suited to their needs.

Method of Infection.

The method of infection of *P. irritans* by *C. pulicis* is purely a contaminative (casual) one, so far as I can ascertain. The faeces of already infected *P. irritans* contain numbers of post-flagellate forms of *C. pulicis* and sometimes also some active flagellates. Other fleas may obtain blood near the spot of skin fouled by their neighbours and thereby ingest some of the post-flagellates. In other cases, as the insects moved about, especially in the confined space of my "flea cages," fluid dejecta contaminated their bodies and legs. In the removal of the offending material, the post-flagellates are brought in contact with the mouth parts and thence find their way into the alimentary canal of the host.

I have seen no indications of hereditary infection. Careful dissection of both male and female genitalia failed to show any form of the parasite therein on examination. Breeding experiments confirmed the absence of *Crithidia* in the eggs. Clean fleas bred clean. Eggs of infected fleas hatched apart from the parents also yielded uninfected stocks. I have never found *C. pulicis* in any situation other than the alimentary tract and dejecta of *P. irritans.*

Some Remarks on Insect Flagellates, especially of the Genus Crithidia.

Earlier in this paper I referred to the fact that bred fleas were used during my investigations and that my blood was the medium on which they fed. This raises two possibilities. According to the

dogmatic hypotheses of certain writers, *C. pulicis* would be a stage of a trypanosome. No trypanosome has ever been observed in my blood, whether examined by smear, thick film or culture, and this examination has been frequently made by highly competent authorities. Further, the extended period over which observations have been made is sufficient to put this supposition entirely out of court.

Again, no sore of any kind has ever developed on my body, though bites of the usual character have been made by the fleas repeatedly. These observations exclude all possibility of *C. pulicis* being a stage or stages in the life-cycle of other flagellates, such as those described by C. Basile, and effectually dispose of the wild hypothesis laid down by a would-be authority, that flagellates of sanguivorous insects must be regarded as stages in the life-cycle of a vertebrate trypanosome.

Also, I have thought it well to investigate fleas from outside sources, and through friends, to whom my best thanks are due, obtained fleas from several places in each of the following counties:—Sussex, Surrey, Hampshire, Gloucestershire, Wiltshire, Somerset, London, Lancashire, Cambridge and Essex. Some of the fleas from each of the above-named counties contained *C. pulicis*. In every case, the parasites presented identically the same morphology and life history as those obtained from my bred *Pulex*. Recently, a criticism (*Bull. Sleeping Sickness Bureau*, May, 1911) was made of a paper by Swingle on the transmission of *T. lewisi* by rat-fleas, in which the critic "presumes" that the fleas used were "wild" fleas, and adds:—"in the present state of our knowledge, in the case of flagellates found in wild insects, it is almost guesswork to say that certain forms are natural flagellates and others stages of blood trypanosomes." Considering the evidence that is steadily accumulating of the occurrence of natural flagellates in blood-sucking insects, the above statement seems a rash and inexact one. Bruce and his colleagues have recently shown that natural flagellates, *Crithidia*, occur in the sanguivorous Tabanid flies suspected of transmitting *Trypanosoma pecorum*, and that the *Crithidia* are flagellates of the insects and not developmental forms of a trypanosome. Wild insects include sanguivorous and non-sanguivorous forms; while the case of "wild" insects feeding on plants is obviously overlooked.

As far back as 1906, Ross published on *Crithidia* in mosquitos in India (observed between 1895 and 1899) and in connection therewith stated (p. 107) "In fact, it was evident that they [*Crithidia*] had been already present in the insects before these were fed on the blood." The sanguivorous habit of an insect is no criterion as to whether a flagellate

occurring therein is a developmental form of a trypanosome, or a true parasite of the insect.

My own work on *C. pulicis* confirms the statement that natural flagellates can occur in sanguivorous insects, and in this case there is certainly no evidence to show that the results obtained on *C. pulicis* from "wild" *P. irritans* could be regarded as "almost guesswork." As with the *Tabanidae*, a natural flagellate of the host is present, and there is not one tittle of evidence to show that *C. pulicis* is other than a natural flagellate of *P. irritans*.

Arm-chair criticism is always easy and after five minutes of wild hypotheses, the march of progress may be endangered for as many years. This has recently been attempted by certain dogmatic individuals who have published compilations regarding trypanosomes, *Crithidia* and *Herpetomonas* without really practical knowledge or first-hand investigation of some of the subjects discussed. But having formed an hypothesis, they then support it blindly. Further, though the hypothesis has been shown by recent research to be untenable, yet they continue to preach the same, and, as with the importunate friend, gain a hearing and obtain followers by their very insistence. The following so obtained is not of the highest order, needless to say, as witness a recent deluded beginner, who in his first essay in the investigation of the flagellates of flies, states of a man who has never published an original paper on *Herpetomonas*, and has merely confused the whole subject in a wordy text-book, that the history of *Herpetomonas* is related in greater detail by him! British Protozoology is in a bad way when original work is neglected, or confused with that of the mere compiler.

While writing this paper, my attention has been drawn to an account published by Mr J. S. Dunkerly on a parasite or parasites which he calls *Leptomonas muscae domesticae*. Most of his paper, however, is devoted to an attempt to discuss the genera *Leptomonas*, *Herpetomonas*, *Crithidia* and *Trypanosoma*. I would not trouble to notice the paper, except that in it the author attempts to establish that *Crithidia* is not a valid genus and he states (p. 649) that "It is with a view to the clearing up of at least one part of the vexed question [nomenclature] that I wish to re-state the following facts in their history." Indeed! This is rather an ambitious task to essay in one's first paper on such a complicated subject as the Flagellates of Insects. But Mr Dunkerly is nothing if not courageous. However, I beg to inform him that a much broader and more accurate view will have to

be taken than the one he has allowed himself to follow. To quote the views of Roubaud, Hartmann and Jollos and Prowazek in full on the one hand, while giving only meagre quotations from Patton, Swingle and Miss Mackinnon on the other, savours more of the methods of the "party politician" than of research for the advancement of Science. I cannot deal with this memoir in detail, for there is scarcely a statement in the paper that is accurate or not misrepresented. Practically all the authorities quoted by Mr Dunkerly have been discussed at length by Patton, Miss Mackinnon and myself, among others, *ad nauseam.*

But I must deal with one remarkable statement in Mr Dunkerly's paper, namely:—"*Crithidia* cannot be applied as a generic name to any form, as it has simply been the name given to two stages in the life history of a *Leptomonas* or to what in other cases are probably stages of Trypanosomes." This is amusing from one who has never published on parasites of the genus *Crithidia,* and, judging from his remarks, would not know a *Crithidia* if he saw it. For instance, he attributes a diagnosis of the genus to Patton, which diagnosis is inaccurate and in a form that Patton never used. According to Mr Dunkerly: "Patton... decided that all uniflagellate parasites of insects with the kinetonucleus anterior to the trophonucleus and without undulating membrane, are to be called *Herpetomonas* and that those having the kinetonucleus posterior to the trophonucleus and possessing an undulating membrane, should receive the generic name of *Crithidia.*" Patton never used the terms "trophonucleus" and "kinetonucleus," and certainly did not say that in *Crithidia* the blepharoplast was always posterior to the nucleus.

Another statement made is also an unworthy one, namely:—"Lühe and Hartmann and Jollos have pointed out that Patton's failure to see the characters observed by Prowazek and others does not prove their non-existence." But Lühe (as listed) wrote in 1906 and Patton in 1908. Further, Patton never denied the existence of certain features in Herpetomonads, but disagreed with their interpretation as given by Prowazek and others—a very different thing. As to Hartmann and Jollos, I am sorry that I cannot seriously consider their second-hand views, for these authors, among other items, place *Piroplasma* (*Babesia*) and the malarial parasites in their "Flagellatenordnung Binucleata," despite lack of supporting evidence.

Mr Dunkerly appears to lay much stress on his edition of Léger's statement that *Crithidia* is "en form (sic) de grain d'orge." Leaving aside the words used in description, let us consider the figures of Léger. If Mr Dunkerly can find any important difference in the figures

of Léger and the figures of young forms of *C. gerridis*, *C. tabani* and *C. melophagia*, given by Patton, Flu, Swingle and myself, he is a mere quibbler. The whole discussion is one of words, words, *ad nauseam*. Mr Dunkerly further attributes to Léger a statement that *Crithidia* is "usually without an undulating membrane." This is an unfortunate inexactitude. Léger (1902) not only recognised "un rudiment de membrane ondulante," but figured it (Léger's Figs. 7–10). All reference also is omitted to Patton's explanation of the differences between Léger's account and his own, these being due to the fact that Léger had not described the fully developed flagellate when he first wrote. The mature *C. fasciculata* is stated by Patton (1908, p. 142) to be "very similar to the adult form (*C. gerridis*) I have described."

To lead up to the conclusion that *Crithidia* is not a valid genus, Mr Dunkerly favours us with what may be described as a crescendo of possibilities, probabilities and certainties, but not a particle of direct evidence or experiment. On p. 648 we read, "However, it *seems* from the evidence of the forms found in *Homalomyia* that the same organism *may be* without an undulating membrane at one stage of its life history, while possessing one at another stage" (the italics are mine). Later p. 651, we read "This...*seems certain* (1) that Léger's original pear-shaped *Crithidia* is only a stage of the *Leptomonas* life-history." Léger did not explicitly describe *Crithidia* as pearshaped in founding the genus. Further, it is a mere assumption that because a *Leptomonas* (in the sense of Chatton and Alilaire) is present in *Homalomyia*, there is really a *Leptomonas* in *Anopheles maculipennis* or in every insect from which *Crithidia* have been obtained.

By the end of his paper, Mr Dunkerly has become even more definite, for on p. 652 we read "Crithidia *cannot* be applied as a generic name to any form, as it has simply been the name given to two stages in the life history of *Leptomonas* or in other cases to what are probably stages of Trypanosoma."

In the first instance, there is no proof whatever that any true Crithidia is a stage or stages in the life history of a *Leptomonas*. Nor does Mr Dunkerly's work aid in that matter in any degree, especially as he is forced to admit (p. 649) that "the low percentage of infections have (sic) prevented the completion of it [the life-cycle] up to the present." Again, dealing with intermediate forms, a statement is made that all stages between the short, truncated forms of *Leptomonas muscae domesticae* and elongated forms with undulating membranes, occur. But the three figures quoted as illustrating intermediate

forms are inadequate and unsatisfactory, for they are all about the same length and merely illustrate, as Mr Dunkerly himself states: "the varying position of the kinetonucleus and the presence of an undulating membrane."

How can dogmatic statements based on the knowledge of part only of the life-cycle of a *Leptomonas* be regarded as finally settling the question of the validity or otherwise of a totally different genus, *Crithidia*? It is a presumption to set forth such statements on such hopelessly inadequate evidence.

With regard to the second part of the statement that *Crithidia* are probably stages of trypanosomes, no definite evidence in support of this statement is forthcoming. I can find nowhere that the conversion of a trypanosome into a true *Crithidia* and of a true *Crithidia* into a veritable trypanosome have been witnessed in the living organisms. *Herpetomonas* and *Crithidia*, considering their definition, have *not* necessarily anything to do with trypanosomes. To introduce the trypanosomes into the discussion of the genera *Herpetomonas* and *Crithidia* is merely and immediately to confuse the issue. Hypotheses as to whether a flagellate is primarily a parasite of an insect or of a vertebrate are purely speculative and can only be treated as such. The opinion of L. Léger in respect to the position of the trypanosomes, is, however, of interest:—"Les trypanosomes du sang, ne représentent qu'une adaptation partielle et secondaire d'un parasite primitivement intestinal ou enterocoelomique d'Invertebré." Having regard to this statement, the discussion of insect flagellates from the trypanosome point of view is commencing at the wrong end and so working backwards.

I think that there is little doubt that Mr Dunkerly has found a Herpetomonad in *Homalomyia canicularis*, belonging to the *Leptomonas* and leptotrypanosomes of Chatton and A. Léger. However, Mr Dunkerly has not got a *Crithidia*, for as Chatton and Léger say, "Elles [leptotrypanosomes] approchent de stade *Crithidia* sans toutefois atteindre." Leptotrypanosomes have the flagellum attached to the body, there is no membrane and the blepharoplast is posterior, conjoined characters which do not occur in *Crithidia*. However, the observations of Miss Mackinnon (1910) on a *Herpetomonas* from a *Homalomyia* are most instructive. Miss Mackinnon finds *Herpetomonas* therein which often exhibit the peculiarity of the flagellum being bent back and "sticking" to the body. May not this account for the phenomenon seen by Chatton and by Dunkerly? It is strange that Mr Dunkerly, although

he refers to Miss Mackinnon's paper, makes no mention of this observation to which the author draws special attention and illustrates by text-figures. That is only another example of the method of partial quotation pursued by those who wish—by methods analogous to those of the party politician—to destroy the genus *Crithidia*.

Again, in a short addendum Mr Dunkerly states that Flu in a recent paper on the parasites of the house fly "in the main" confirms "the chief points emphasised" by him. I am sorry that I cannot agree, for *Crithidia* is recognised by Flu as a genus, is defined by him and is kept separate from *Leptomonas*.

More earnest endeavour to work out the life history of flagellates such as *Leptomonas* is emphatically needed. At present, the flagellate form only is at all adequately dealt with, and until the complete cycle has been obtained, hypotheses built on knowledge of the flagellate stage only cannot but be somewhat insecure.

In conclusion, one is impelled to wonder whether research in Protozoology is to be conducted merely on the lines of party politics instead of the advancement of knowledge. Perhaps the seeming advocates of the former policy will re-consider their position. Apparently, Dr Woodcock, having written a thoroughly confused account of the Haemoflagellates, is now to be allowed to edit the work of other investigators more capable than himself, and to support Dr Woodcock we are to have the inexperienced efforts of his henchmen, like Mr Dunkerly, who seems in the domain of Protozoology to be essaying to fly in an aeroplane before he knows how to walk. The whole procedure is unworthy of British Protozoology and contributes nothing to the extension of knowledge.

Common sense dictates that the word of the practical worker is of more value than that of the "arm-chair critic." The statements of practical investigators like Patton, Flu and Swingle must be of infinitely more value than the hypotheses of Woodcock, Hartmann and others who have not themselves investigated *Crithidia*. It is amusing to note that apparently the only writers who wish to destroy the validity of the genus *Crithidia* are those who have not published researches on the same, and so cannot be expected to have a first-hand knowledge of the genus. We have to work out complete life-cycles if we are to go forward, and all the wrangling over nomenclature and evolution will be of no avail in the end. However, time will tell.

Summary.

1. *Crithidia pulicis*, n. sp., is a parasite of the alimentary tract of the human flea, *Pulex irritans*.

2. The fleas used in this investigation were bred to the third generation in special "flea cages" on the human body. "Wild" fleas were examined and yielded the same parasite. Both larvae and adult fleas were examined.

3. *C. pulicis* exhibits pre-flagellate, flagellate and post-flagellate stages in its life history and these phases gradually develop, the one from the other.

4. The flagellate form is very active, movement being accomplished by means of the myonemes of the membrane and body.

5. Pre-flagellates (figs. 1–8) are oval bodies, from 2·3 μ to 7 μ long by 1·5 μ to 4·5 μ broad. The nucleus contains much chromatin and a small karyosome may be present. The blepharoplast is large. The flagellum develops from a chromatophile area. The rate of acquisition of flagellum and membrane varies in individual parasites. Division rosettes are frequent. The pre-flagellates have a somewhat frail appearance.

6. The flagellates of *C. pulicis* (figs. 9–17) are 26 μ to 65 μ long. Their cytoplasm is richly but finely granular, the nucleus well marked, the blepharoplast large, showing slight variations in shape. Chromidia may be present. The undulating membrane is large, well marked and possesses myonemes which can be detected in the living organism. The free flagellum is relatively short.

7. Post-flagellates (figs. 23–36) are from 3 μ to 6 μ by 2·0 μ to 4·6 μ. They occur in the rectum and dejecta of the fleas. They are produced by concentration of the protoplasm round the nucleus and blepharoplast with absorption of the membrane and flagellum and finally the production of a thin varnish-like coat.

8. Longitudinal division (figs. 15–22) is the method of multiplication. It occurs in the pre-flagellate and flagellate stages. It may be symmetrical or asymmetric. Division is initiated by that of the blepharoplast, followed by that of the flagellum, membrane and nucleus and finally the body cytoplasm. Two types of asymmetric division have been observed.

9. The method of infection is contaminative, the post-flagellates in the faeces being the source of infection. There is no evidence of hereditary infection.

10. *Crithidia pulicis* is a member of the genus *Crithidia*, and is a true parasite of the insect, *Pulex irritans*.

REFERENCES.

Further references will be found at the ends of some of the papers quoted.

Basile, C. (1910–11). Sulla Leishmaniosi et sul suo modo di trasmissione. *Rendiconti de R. Accad. d. Lincei*, t. xix. 20 Nov. 1910, pp. 523–527; t. xx. 8 Jan. 1911, pp. 50, 51; 19 Feb. 1911, pp. 50, 51; 19 March 1911, 7 pp.

Bruce, D., Hamerton, A. E. and Bateman, H. R. (iv. 1911). Experiments to ascertain if certain Tabanidae act as the carriers of *Trypanosoma pecorum*. *Proc. Roy. Soc.* B. 83, pp. 349–358.

Bruce, D., Hamerton, A. E. and Mackie, F. P. (v. 1911). Further researches on the development of *Trypanosoma gambiense* in *Glossina palpalis*. *Proc. Roy. Soc.* B. 83, pp. 513–527.

Chatton, E. et Alilaire, E. (1908). Coexistence d'un *Leptomonas* (*Herpetomonas*) et d'un Trypanosoma chez un muscide non vulnérant, *Drosophila confusa* Staeger. *Compt. Rend. Soc. Biol.* lxiv. pp. 1004–1006.

Chatton, E. et Léger, A. (1911). Eutrypanosomes, *Leptomonas* et Leptotrypanosomes chez *Drosophila confusa* Staeger (Muscide). *Compt. Rend. Soc. Biol.* lxx. pp. 34–36.

—— (1911). Sur quelques *Leptomonas* de Muscides et leurs Leptotrypanosomes. *Compt. Rend. Soc. Biol.* lxx. pp. 120–122.

Dunkerly, J. S. (vi. 1911). On some stages in the life-history of *Leptomonas muscae domesticae*, with some remarks on the Relationships of the Flagellate Parasites of Insects. *Quart. Journ. Microsc. Sci.* lvi. pp. 645–655.

Flu, P. C. (1908). Ueber die Flagellaten im Darm von *Melophagus ovinus*. *Arch. f. Protistenk.* xii. pp. 147–153.

—— (1911). Studien über die im Darm von Stubenfliege, *Musca domestica*, vorkommenden protozoären Gebilde. *Centralbl. f. Bakt. etc.* lvii. i Abt. Originale, pp. 522–535.

Léger, L. (1902). Sur un flagellé parasite de l'*Anopheles maculipennis*. *Compt. Rend. Soc. Biol.* liv. pp. 354–356, 10 figs.

—— (1904). Sur les affinités de *l'Herpetomonas subulata* et la phylogenie des Trypanosomes. *Compt. Rend. Soc. Biol.* lvii. pp. 615–617.

Mackinnon, D. (1910). Herpetomonads from the Alimentary Tract of certain dung-flies. *Parasitology*, iii. pp. 255–274.

Patton, W. S. (1907). Preliminary note on the development of a species of *Herpetomonas* found in *Culex pipiens*. *Brit. Med. Journ.* ii. pp. 78–80.

—— (1908). The Life-cycle of a species of *Crithidia* parasitic in the intestinal tract of *Gerris fossarum*, Fabr. *Arch. f. Protistenk.* xii. pp. 131–146.

Patton, W. S. (1908). *Herpetomonas lygaei. Arch. f. Protistenk.* xiii. pp. 1–18.

—— (1909). A critical review of our present knowledge of the Haemoflagellates and allied forms. *Parasitology*, ii. pp. 91–143.

—— (1909). The Life-Cycle of a species of *Crithidia* parasitic in the intestinal tracts of *Tabanus hilarius* and *Tabanus* sp. *Arch. f. Protistenk.* xv. pp. 333–362.

Patton, W. S. and Strickland, C. (1908). A critical review of the Relation of Blood-sucking Invertebrates to the Life-cycles of the Trypanosomes of Vertebrates, etc. *Parasitology*, i. pp. 322–346.

Porter, A. (1909). The Morphology and Life-history of *Crithidia gerridis*, as found in the British water-bug, *Gerris paludum*. *Parasitology*, ii. pp. 348–366.

—— (1909). The Life-cycle of *Herpetomonas jaculum* (Léger), parasitic in the Alimentary Tract of *Nepa cinerea*. *Parasitology*, ii. pp. 367–391.

—— (1910). The Structure and Life-history of *Crithidia melophagia* (Flu), an Endoparasite of the Sheep Ked, *Melophagus ovinus*. *Quart. Journ. Microsc. Sci.* lv. pp. 189–224.

—— (1911). Some remarks on the Genera *Crithidia*, *Herpetomonas* and *Trypanosoma*. *Parasitology*, iv. pp. 22–23.

—— (1911). Some remarks on the Genera *Crithidia*, *Herpetomonas* and *Trypanosoma*, and Dr Woodcock's views thereon. *Parasitology*, iv. pp. 154–163.

Ross, R. (1906). Notes on the Parasites of Mosquitoes found in India between 1895 and 1899. *Journ. Hyg.* vi. pp. 101–108.

Sangiorgi, G. (1911). Sulla presenza di forme di *Leishmania infantum* (Nicolle) nella pulce (*Pulex serraticeps*) dei cani randagi di Catania. *Pathologica*, iii. pp. 23–24; 89–90.

Swingle, L. D. (1911). The Transmission of *Trypanosoma lewisi* by rat-fleas (*Ceratophyllus* sp. and *Pulex* sp.), with short descriptions of three new Herpetomonads. *Journ. Infect. Diseases*, viii. pp. 125–146.

Werner, H. (1908). Ueber eine eingeisselige Flagellatenform im Darm der Stubenfliege. *Arch. f. Protistenk.* xiii. pp. 19–22.

EXPLANATION OF PLATE X.

All figures were outlined with the Abbé-Zeiss camera lucida, using a 2 mm. apochromatic (Zeiss) or $\frac{1}{12}$ inch achromatic (Zeiss) objective, and compensating oculars 8 and 12. The magnification is approximately 1300 diameters except where otherwise stated.

Figs. 1–8. Pre-flagellate stages of *Crithidia pulicis*.

Fig. 1. Large pre-flagellate showing well-marked nucleus with karyosome and distinct blepharoplast.

Fig. 2. Smaller pre-flagellate × 1950.

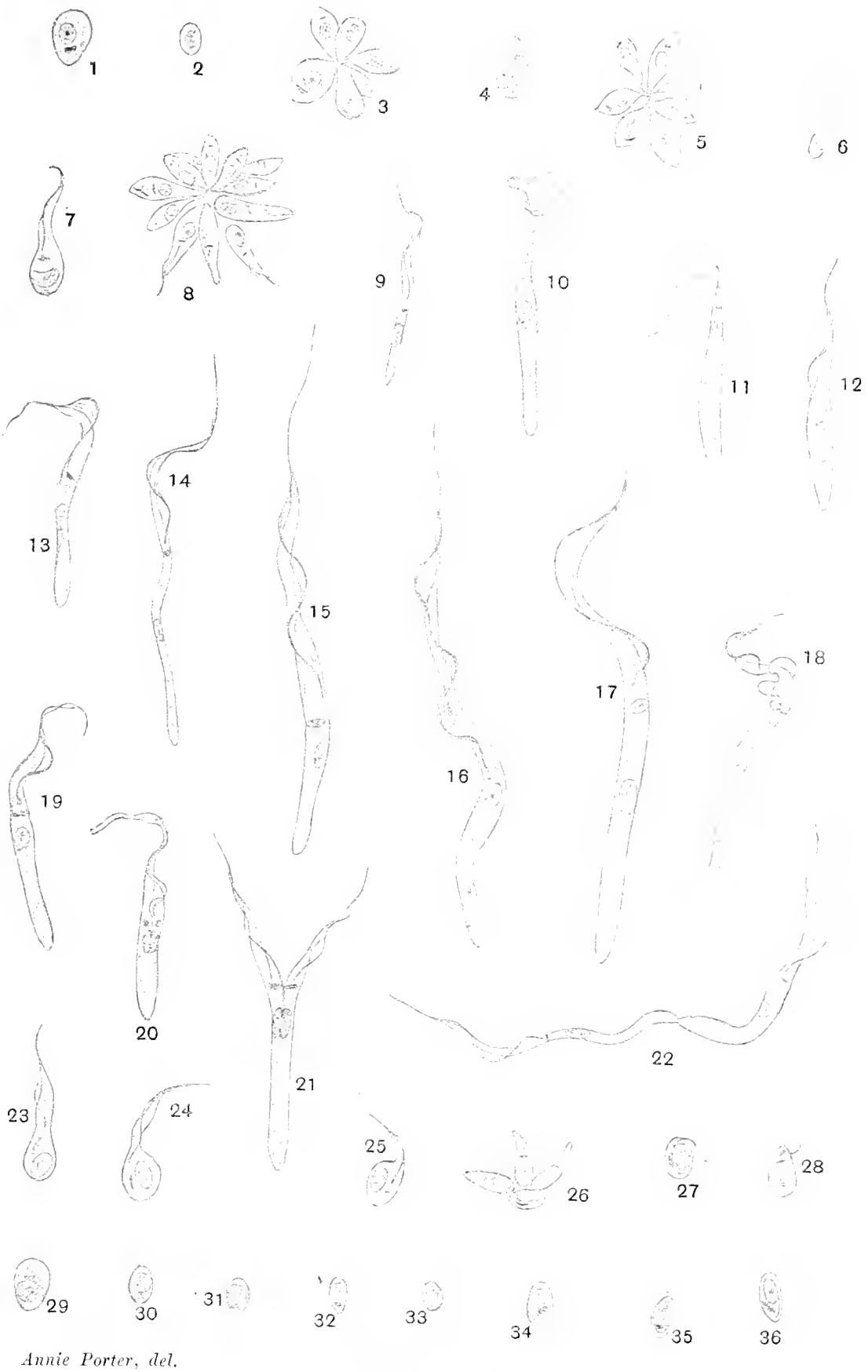
Fig. 3. Rosette of pre-flagellates, beginning to elongate.

Fig. 4. Pre-flagellate, showing commencement of differentiation of flagellum.

Fig. 5. Rosettes of pre-flagellates showing slight variation in the shape and position of the blepharoplast and in the extent of elongation.

Figs. 6, 7. Elongating forms.

Fig. 8. Rosette, showing unequal rate of development of the pre-flagellates.
Figs. 9–17. Flagellates of *C. pulicis*.
Fig. 9. Small flagellate, showing nucleus, well-marked blepharoplast and body myoneme.
Fig. 10. Larger parasite, showing oblique blepharoplast and very chromatoid nucleus.
Fig. 11. Flagellate with broad posterior end.
Fig. 12. Broad form.
Fig. 13. Parasite showing twist of the posterior end of body. Such an appearance is often seen in life.
Fig. 14. Very narrow form, a division product of such forms as shown in figs. 15–17.
Figs. 15–17. Large flagellates, showing the very characteristic prominent membrane with myonemes. These forms are common.
Figs. 18–22. Stages in the division of *C. pulicis*.
Fig. 18. Broad form showing beginning of division of the blepharoplast.
Fig. 19. Shows parasite with dumb-bell shaped blepharoplast and flagellum beginning to split.
Fig. 20. Parasite showing complete division of blepharoplast and membrane, nucleus constricted.
Fig. 21. Separation of two daughter forms in symmetrical fission.
Fig. 22. Asymmetric division of *C. pulicis*. Separation of daughter forms almost complete.
Figs. 23–36. Post-flagellate stages of *C. pulicis*.
Figs. 23–25. Shortening forms showing concentration of protoplasm round the nucleus and blepharoplast and retraction of the flagellum.
Fig. 26. Cluster of four parasites, one of which has become the typical post-flagellate, the other three being not quite so advanced.
Figs. 27, 28. Almost complete assumption of the post-flagellate form. Fig. 28 × 1950.
Figs. 29–33. Post-flagellates showing variation in size.
Figs. 34–36. Post-flagellates in which the contents are somewhat shrunken away from the thin varnish-like wall.



Annie Porter, del.

Crithidia pulicis, n.sp.

THE NATURE AND SPECIFICITY OF NEGRI BODIES.

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With Plate XI and 2 Text-figures.

By many observers Negri bodies have been considered to be parasitic in nature (Negri, 1903; Babes, 1907). Williams and Lowden (1906) go further and describe the life cycle of these bodies, regarding them as belonging to the Sporozoa, and give to them the name of *Neuroryctes hydrophobiae.* Calkins (1910), although he states that the parasitic nature of these bodies is not proven, still evidently inclines to that view. In his criticism of the work of Williams and Lowden he comes to differ from them as regards the classification of the supposed organisms. He thinks that their variable forms, the uninucleate condition, the occurrence of a state of distributed chromatin, and the budding phenomenon, are characteristic not of Sporozoa but of parasitic Rhizopods. His opinion is that the distributed chromatin masses in the Negri bodies are in all probability representative of the idiochromidia which are so characteristic of Rhizopods.

On the other hand, many have doubted the parasitic nature of these bodies, as well as their specificity. Poor (1906) described in the tetanized guinea-pig, numerous very small inclusions resembling minute Negri bodies situated in the interior of the nucleus of the Ammon-horn cells.

Lina Luzzani (1905) of Pavia examined the central nervous system of twelve cats suspected of rabies. In only two of them was she able to

find typical Negri bodies and these two were proved to be rabid experimentally. In seven, the experimental results were negative both as regards the microscopical and the animal test. In the remaining three, the animal test was positive, whilst microscopically minute bodies ($\frac{1}{3}$ to $\frac{1}{2}$ μ) resembling Negri bodies were seen. In order to clear up this last point she examined the cerebellum and hippocampus major of normal cats and those which had died of diseases other than rabies. She found that these parts in the cat, both in the normal and diseased state may exhibit forms resembling typical Negri bodies. In conclusion she states that, to be certain of the diagnosis of rabies in this animal it is necessary to carry out the experimental tests as well as the microscopic. Pace (1904) has found Negri-like bodies in three persons who died of old age, cerebral embolism, and aortic disease respectively.

Babes (1906) had described, in cases dying from arsenical poisoning, bodies very analogous to Negri bodies in the spinal ganglion cells.

Carlos França (1906) has pointed out that the presence of Negri bodies is one highly correlated with rabies and that it is a condition almost pathognomonic for this disease. But he states that on the one hand they are not invariably present in the nervous system of rabid animals and on the other may occasionally be seen in men and animals who have not succumbed to this infection. A great deal of work has been done on bodies which in many respects resemble those met with in rabies. These occur for example in Variola, Chicken pox, Molluscum contagiosum, Trachoma etc., and are named variously after their discoverers (Guarnieri, Prowazek etc. or in a way to suggest a parasitic nature). The term Chlamydozoa (Prowazek) is very generally used as a family name for this group of supposed organisms. A general description of them is given by Hartmann (1910) which we may reproduce here.

"To begin with large granules are met with in the infected cell for the most part near the nucleus ('initial granules'—first described by Herzog and Lindner), which in division give rise to dumb-bell-shaped figures. Later these granules are surrounded by reaction-products (nucleolar substance). By continued division a large number of smaller granules are produced—the so-called 'elementary granules.' These latter together with the extruded reaction-products of the nucleus form a 'cell-inclusion' which is usually closely applied like a cap to the nucleus. Through the pressure of these elementary granules, regarded as possessed of the properties of parasites, the cell is stimulated to extrude a reaction-product which envelops them like a mantle. On this

account Prowazek has given these parasites the name Chlamydozoa. With further multiplication of the 'elementary granules' the 'cell-inclusion' breaks up leaving only débris, and the whole cell becomes completely filled by the 'elementary granules.' Hartmann and Leber observed dumb-bell-shaped division forms among 'the elementary granules.'"

With this general statement we may now proceed to our own observations on Negri bodies.

To test the specificity of Negri bodies, we carried out a number of experiments on normal guinea-pigs with the following substances:—

(i) Russell's viper venom.

(ii) Cobra vemon.

(iii) Tetanus toxin.

(iv) Living *Bacillus pyocyaneus* emulsion.

(v) Brain matter of a healthy dog which had been preserved in glycerine for 72 hours.

(i) Some 20 experiments are carried out with *Russell's viper venom.*

In two of them out of the 20 we were able to find bodies varying from 1 to 6 μ in size in the cells of the fascia dentata although not in the ganglionic layer of the Ammon horn. Some of these bodies were identical as regards their staining reaction, situation and character with those of the Negri bodies, as found in this animal, whilst others did not give these reactions and were probably cytoplasmic in origin (Pl. XI, fig. 1). The Negri-like bodies were very few in number and required a good deal of searching for, but in the Purkinje cells of one of these guinea-pigs they were fairly large and numerous.

In these experiments doses of 0·001 to 0·0005 milligrammes per 500 grammes weight of guinea-pig were injected subcutaneously in the region of the upper abdomen. Both the guinea-pigs showing these bodies survived for over 48 hours; most of the others died earlier.

(ii) *Cobra venom*: 20 experiments were performed on guinea-pigs, the minimal lethal dose being found to be 0·0001 gramme per 500 grammes of guinea-pig weight. Sections were examined from the hippocampus major and cerebellum of all these animals, but no Negri bodies were seen. In some of the large ganglion cells ovoid masses of cytoplasmic material were observed, which did not stain with Mann's method and were probably of the nature of the plastids.

(iii) *Tetanus Toxin.* In these experiments we were unable to find anything that resembled Negri bodies. The same number of tests were carried out with this toxin as with Russell's viper venom. Sir David Semple, M.D., Director of the Central Research Institute, Kasauli, also kindly supplied us with material from guinea-pigs suffering from chronic tetanus. We were unable to confirm Poor's observations on the bodies described by him as occurring in the nucleus. The only structures we saw in these nuclei other than true nucleoli, were the normal chromatin nodes.

As the action of this toxin is exerted chiefly on the cells of the anterior horn of the spinal cord, we did not expect to see these Negri-like bodies in the hippocampus major or cerebellum; nor did we do so. We did not consider it necessary to conduct experiments with strychnine, as this drug has a similar action to tetanus on the anterior cornual cells.

(iv) *Living Bacillus pyocyaneus emulsion.* These experiments were conducted for a totally different purpose from that with which this paper is concerned, namely to show that a septicaemia due to this bacillus occurs as a natural disease in dogs and that its symptoms closely simulate rabies. This work is to be shortly published by us. In the experiments that were performed we were able in two cases to find Negri-like bodies; these were found in the rabbit and were quite typical of Negri bodies as seen in these animals.

(v) *Normal cerebral matter of a dog* was preserved for 72 hours in glycerine and then made into a thick emulsion; 1 c.c. was injected into the neck muscles of a guinea-pig. Fifteen animals were experimented with and fourteen of these died; one has survived up to date. In five of them control cultures were made from the heart blood, brain and liver and all these remained sterile.

In one case bodies were seen resembling Negri bodies, but they were few in number and only seen in the fascia dentata. In another case nucleolar fragmentation and extrusion of the fragmented particles were seen, but the particles still retained the nucleolar staining reaction. The other guinea-pigs did not show anything abnormal.

A chameleon was given 1 c.c. of 1–100 living fixed virus emulsion. It survived for over a month and during the whole of this time apparently did not eat any food offered to it. On examination numerous large undoubted Negri bodies were seen in the cortical cells of the cerebrum. Whether these were due to the action of the fixed virus or not we cannot say. But in mammals fixed virus does not usually give rise to Negri bodies.

From the above facts it can be seen that Negri bodies are not truly specific of rabies. At the same time we must state that in none of the above experiments have the Negri bodies been either so numerous or so large as those seen in rabies. As far as these experiments go we should be inclined to assert that—specificity apart—there is undoubtedly a very high correlation between the presence of numerous Negri bodies and rabies. Thus their presence in marked numbers forms a valuable if not absolutely certain test for rabies. If they are few in number or absent, no definite conclusions can be drawn.

Having shown that Negri bodies are not truly specific for rabies, we may now proceed to study their so-called life cycle as described by Williams and Lowden in the dog. If Negri bodies were of the nature of parasites, we would expect them to be similar in structure and development, whatever species of animal acted as host. From this point of view the following animals were examined: man, horse, mule, cow, goat, monkey, rabbit, guinea-pig, dog and jackal. The tissues were hardened in Zenker's fluid, spirit, formalin, picro-formalin and acetic bichromate mixtures; Zenker's fluid was found to be the most satisfactory of them all. The tissue was embedded in paraffin wax, and the sections stained either by Mann's methyl-blue eosin (long method) or by iron haematoxylin (Mallory) and in the latter case counter-stained by the picro-fuchsin mixture of Van Gieson, or Bordeaux red.

The physico-chemistry of staining reactions is a particularly difficult subject. We do not propose to enter upon it here. But it is necessary to give some idea of the alterations in staining reactions of the bodies we are about to describe. This is particularly necessary in order that our terminology may be rendered exact. Normal nerve cells show when stained by Mann's long method a red nucleolus, and with Mallory's iron haematoxylin a black nucleolus. Now, if the cell be a normal one, counter-staining with Van Gieson's stain or Bordeaux red does not give rise to any alteration in the colour of the nucleolus. In nerve cells of brains affected with rabies the case is otherwise. With Mann's stain the nucleolus stains red or reddish yellow and Negri bodies stain red. With iron-haematoxylin and Van Gieson's stain the nucleolus may stain black, or, more usually, it stains greenish or terracotta. Precisely similar staining reactions are seen in the Negri bodies. The larger Negri bodies may show in addition to the green or terracotta some black dots (iron stain). It would seem as if in rabies-brains the iron staining of the nucleolus was more easily removed by the decolourisation employed than in normal brains, thus allowing the

nucleolus to be coloured by the counter-stains. These statements are based on an examination of a large number of sections—upwards of a thousand. With this explanation, it is possible for us to use the terms iron staining (black), Mann's stain (red), Van Gieson's stain (green and terracotta) without ambiguity and so avoid a resort to the more common and at the same time more controvertible terms acidophile, basophile, cytoplasmic staining etc.

(i) *Man.* We were fortunate in being able to examine six cases all told, three of which Major Cornwall, I.M.S., Director of the Pasteur Institute, Coonoor, kindly sent us. The Negri bodies seen were small in size, scanty in number, and for the most part occurred only in the large ganglion cells of the Ammon horn. They vary in size from 1–6 μ and appear homogeneous. Sometimes a vacuole is seen in them. As a rule these bodies in man have an affinity for Mann's or Van Gieson's stains. In the multipolar cells of the cortex, instead of Negri bodies, large irregularly shaped masses may be seen (Pl. XI, fig. 2) lying usually at the base of the cells. They stain with difficulty and appear yellow with the Van Gieson mixture, and also with Mann's stain. It seems likely that neither of the stains referred to is able to colour these masses. Examined under a high power, these masses appear to consist of an aggregation of small vesicles and sometimes they distend the nerve cell so that the nucleus may scarcely be discernible. The nucleolus of these cortical cells is single, and, by Mann's method of staining, shows an outer red ring and a centre, probably fluid, which is yellow in colour like the extra-nuclear mass. These masses were first described by Major Cornwall, I.M.S. In a private communication we had from him lately he informs us that he does not now regard these masses as specific of rabies in man, because he has found them in diseased conditions other than rabies.

(ii) *Horse.* The Negri bodies vary in size up to 10 μ, they are fairly numerous and sometimes contain a primary and even a secondary vacuole. Their staining reactions are the same as in man. In the cortex of a definitely rabid mule, similar yellow masses to those described by Major Cornwall, I.M.S., were found in the pyramidal cells.

(iii) *Bullock.* The Negri bodies are very large and numerous and are seen in the cells of the hippocampus major, cortex and cerebellum (Pl. XI, figs. 3 and 4). There are often several in a single cell and they vary in size from 4 μ to 20 μ. They are round or ovoid in shape. Frequently they are situated near the nucleus, and, when they are in this region, they are indented by it. The small Negri bodies show a

well marked central vacuole and as they grow large a secondary vacuole appears. In the largest Negri bodies a large central vacuole, surrounded by numerous secondary ones, is to be seen (Pl. XI, fig. 4). The nucleolus of these nerve cells may be multiple and generally takes Mann's stain or as the case may be Van Gieson's.

(iv) *Goat.* The Negri bodies are small (1 to 6 μ) and scattered among the ganglion cells of the Ammon horn. They are ovoid in shape and usually exhibit one vacuole. In the cells of the fascia dentata, of this animal as well as the bullock, small Negri bodies are seen varying from 1 to 4 μ in size and round in shape.

(v) *Monkey.* The Negri bodies are small (1 to 6 μ) and homogeneous. In a rabies-infected monkey that died on the 12th day of incubation from intussusception (*i.e.* a very early case of rabies) the Negri bodies were situated close to the nuclei, giving an appearance as if they had just been extruded from it. The tangential and circumferential fibres of the nerve cells were fragmented, giving rise to a fine punctate appearance of the extra-cellular ground substance. It is this punctate appearance, due to fragmentation of fibres, which may explain the fine chromatoid peppering found in rabies brains by some observers. In the cerebellum, the Negri bodies were large (6 to 12 μ). They all stained red with the Van Gieson mixture. No yellow masses were seen in the cortical cells.

(vi) *Rabbit.* In this animal the Negri bodies are all exceedingly small, varying from $\frac{1}{4}$ to 2 μ in size and generally 2 to 5 are seen in the cell. They appear homogeneous in structure, and stain by Van Gieson's method (Pl. XI, figs. 5, 6). The nucleoli of the cells are markedly fragmented, and generally stain red or terracotta.

(vii) *Guinea-pig.* This is the animal *par excellence* in which to study Negri bodies, and it was the observation of the appearances found in test animals, that led us to commence this study on the nature of these bodies. Here one at once recognised the close similarity in appearance of Negri bodies to the cap-shaped archoplasmic vesicle found in the sperm cells of this animal. Our studies of the Negri bodies in guinea-pigs were especially large and extended to over 100 animals. In the ganglion cells of the hippocampus major, Negri bodies, very large for the size of the animal, are seen, varying in size from 4 to 16 μ (Pl. XI, fig. 9). They are round or ovoid and usually situated near the nucleus. With careful focussing it will be found that they are indented on the nuclear side, and appear as if they gripped the nucleus. The larger ones may show an indefinite lighter stained centre, but no

arrangement of primary and secondary vesicles is evident. With the Van Gieson mixture they usually stain greenish, but take the usual red colouration with Mann's stain. The nucleus behaves, as regards its staining reactions, in precisely the same way. In the fascia dentata (Pl. XI, fig. 7) the Negri bodies are about 2 to 6 μ in length and many of them distinctly cap-shaped; on careful focussing the base of the cap is found to be indented by the nucleus.

In the cerebellum they are usually large in size, irregularly vacuolated, and stain by Van Gieson. In the cortex they are numerous and scattered (Pl. XI, fig. 8). The guinea-pig, goat and bullock are the only animals of this series, in which we have seen Negri bodies in the fascia dentata.

(viii) *Dog.* The Negri bodies in this animal have often been described in detail. The only facts to be mentioned with regard to them are that they are often found in the dendritic processes of the nerve cells, and in this situation are elongated, showing that their contents are plastic. They usually stain terracotta but occasionally greenish with Van Gieson. The nucleolus almost always stains greenish.

(ix) *Jackal.* In this animal the Negri bodies closely simulate those seen in the dog as regards their size, shape, and staining reactions. They were more numerous, and the majority of them stained green with Van Gieson. We are disposed to believe that the younger Negri bodies in these various animals take the green stain and the older take the terracotta stain with Van Gieson.

From the foregoing we see that these Negri bodies vary in shape, size, and staining reactions in the different animals examined, whilst remaining constant for the same animal. In the rabbit they are extremely small, 1 to 2 μ in size, non-vacuolated and stain terracotta (Pl. XI, figs. 5, 6). In the bullock they are extremely large, sometimes as much as 20 μ, stain terracotta, and show vacuolation early in their development (Pl. XI, fig. 4). In most of the animals examined they stained terracotta with the Van Gieson mixture, but particularly in the guinea-pig, though also in the jackal and dog, many of them stain green with this stain. The variations in the staining reaction of the nucleoli, as well as of these bodies, possibly denote a difference in chemical composition or reaction, but we state this with some reserve, as anyone who has worked on nucleolar structures knows the difficulty of interpretation of these reactions.

In man, horse and mule the yellow masses, described by Major Cornwall, I.M.S., were seen; in the other animals examined they were absent.

In one of our experiments, a virus obtained from a man's brain was inoculated subdurally into two monkeys, two goats, a cow-calf, a rabbit, a guinea-pig and intramuscularly into a white mouse. The latter survived, but all the other animals died of rabies and showed the Negri bodies particular to the species. An emulsion of the brain of the rabid calf was inoculated subdurally into a rabbit. Large Negri bodies had been found in the cow-calf (Pl. XI, figs. 3, 4). When the rabbit died the typical small Negri bodies of that animal were seen (Pl. XI, figs. 5, 6). The incubation-period did not differ significantly in the rabbit and calf, for death took place on the 16*th* and 15*th* day respectively—that is to say we may assume the Negri bodies in each to be of about the same age. This experiment, together with our other observations, would indicate that the size etc. of the Negri body do not depend upon the virus, but rather on the kind of animal. This points to a dependence of formation on the response of the nerve cell. In fact we may go further and say that we are now able, for the above animals, to diagnose in an unknown section the particular mammal concerned by such characters alone as the size, shape, vacuolation and staining reaction of the Negri body. Moreover there appears to be some association between the size of the cell and the size of the Negri body. The above facts are not in accordance with conceptions of the parasitic nature of these bodies. If they were parasites we should expect them to be uniform in structure etc., whatever the animal which acted as host. Moreover they cannot be specific parasites and yet be found in other diseases than rabies. The denial of their parasitic nature does not affect the question of their importance for the diagnosis of rabies—a fact which has been amply proved. It still remains to be explained what these bodies really are. We consider that the nucleolus plays a large part in the formation of Negri bodies and therefore a consideration of the structure and variations of the nucleolus will not be out of place here.

If we study the nucleolus of a normal ganglion cell of the central nervous system, we will find that it consists of a large single spherical body, which apparently is suspended in the nuclear network and shows no differentiation of structure. It is not composed of chromatin proper, and stains usually an intense black with the iron haematoxylin method of Mallory and a brilliant red by the methyl-blue eosin method of Mann. The peripheral portion is probably of a different composition to the fluid centre, and hence often stains a deeper black than the centre and so the bull's eye effect which was first described by Heidenhain (1882)

is produced. The neuroglial cells do not exhibit the true nucleolus, but numerous chromatin nucleoli are seen. These false nucleoli are of a different type and are generally known as "Netz-Knoten," chromatin nucleoli or karyosomes. They are condensed portions of the chromatin network and exhibit all the characters of chromatin. They are generally multiple, irregular in shape, and can hardly be distinguished except by size from the nodes of the chromatin network. The exact relationship between these two forms—true and false nucleoli—is as yet far from being certain, owing to the fact that variations occur in the staining reactions of the true nucleolus so as to render it not improbable that intermediary forms may exist which may represent an actual transition of one to the other (Wilson 1906).

In contrast to the nucleolus of the normal ganglion cell, the germinal spot (or true nucleolus) of the ovum is generally of a very large size (*e.g.* in Echinoderm eggs). It appears as a large single spherical body. As a rule the single nucleolus gives rise to several nucleoli with the growth of the ovum. In the ovum of amphibians and reptiles they may be counted by hundreds. Fleming (1882) has described two types of general nucleoli in the ovum (*a*) the principal nucleolus and, (*b*) the accessory nucleoli. These differ widely in staining reaction, and it is still *sub judice* whether they represent the plasmosomes or karyosomes of tissue cells. The principal nucleolus stains deeply like chromatin and yet differs widely from the chromatin network and does not play any part in the contribution to the formation of the chromosomes. It is vacuolated and sometimes assumes the form of a hollow vesicle. The accessory nucleoli on the other hand are generally coloured by plasma stains and thus resemble the plasmosomes of the cytoplasm: they are generally multiple and appear to arise during the growth of the ovum. As a rule no visible relationship is evident between these two kinds of nucleoli, but in annelids they are closely united to form a compound body. During the early pro-phase and meta-phase of mitosis, the principal nucleolus may be cast out bodily from the dividing nucleus, *e.g.* in the parasitic annelid Myzostoma. In the copepod (Heterocope) during the mitosis of its ovum, the principal nucleolus degenerates as soon as the germinal vesicular membrane has disappeared. Montgomery (1898) in his study of the subcutaneous glands of *Piscicola*, found that their cell-nuclei at first contained a single nucleolus. During growth of the cell the nucleolus undergoes fragmentation and gives rise to about 300 nucleoli one of which remains behind and the remainder migrate into the cytoplasm. Chubb (1906), in his paper on the growth of the

oöcyte in *Antedon bifida*, states that "throughout the growth of the oöcyte the nucleolus intermittently discharges groups of deeply staining basophile granules into the cytoplasm. In the young oöcyte these nucleolar spherules remain unchanged and slowly accumulate in the cytoplasm where they form small groups near the germinal vesicle. In slightly older oöcytes, the increased fluidity of the cytoplasm (which results from the progressive accumulation of the metaplastic material in preparation for yolk formation) causes the discharged nucleolar matter to lose its spherical form and to diffuse into the neighbouring cytoplasm. The deeply stained area of protoplasm to which this diffusion gives rise is the yolk nucleus. The yolk nucleus assumes the form of a concavo-convex lens and embraces the germinal vesicle. During yolk formation,

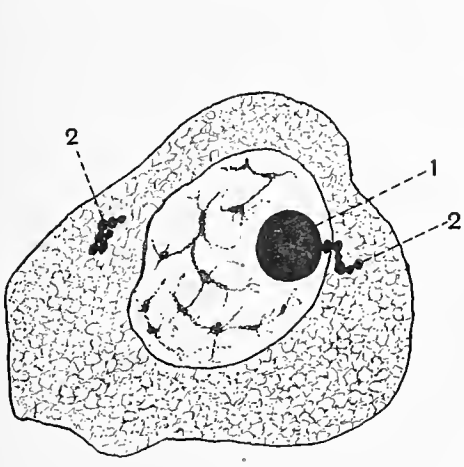


Fig. 1.

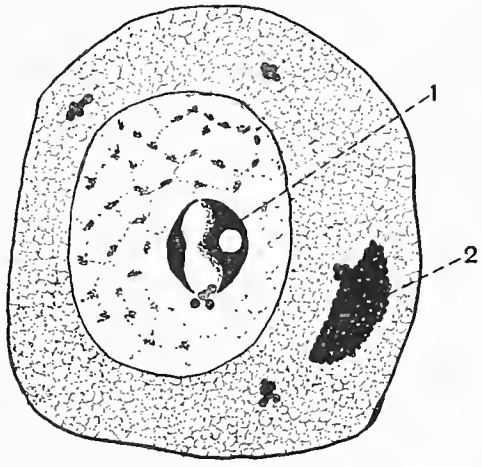


Fig. 2.

Fig. 1. 1. Nucleolus. 2. Discharged nucleolar particles.
Fig. 2. 1. Nucleolus discharging nucleolar granules. 2. The yolk nucleus.
(From Chubb, reproduced in Adami's *General Pathology*.)

the yolk nucleus passes to the periphery of the ovum, a migration also shared by the peripheral nucleolar spherules. The yolk nucleus is therefore a region of the cytoplasm into which waste, discharged from the nucleolus has taken place." (Text-figs. 1, 2.) Macallum (1891) in his study of the germinal vesicles of the developing ova of the *Necturus*, found at a certain stage of development, that the nucleoli occurred at the periphery of the germinal vesicle, in apposition with the vesicular membrane. The nucleoli vary in size but are somewhat spherical in shape. At this stage of development no yolk spherules are seen. By employing indigo-carmine as a stain he found that these peripheral nucleoli took on a blue stain whilst the nucleolus and cytoplasm were stained red. At a later stage, when yolk formation had occurred, the

peripheral granules were smaller and the yolk spherules stained blue. Sometimes it was possible to see around individual granules a certain amount of blue staining of karyoplasm, indicating in his opinion that the nucleoli were generating substances which were diffusing through the vesicular membrane to give rise to the yolk. Steinhaus (1890) in his study of the pancreatic cells, found the nuclei to possess safranin-staining nucleoli and on double staining with haematoxylin the nucleoli remained red, whilst the rest of the nucleus took on the reddish purple colour of haematoxylin. As the nucleolus lost its safranin-staining affinity, the cytoplasm acquired safranin granules. From this he inferred that the nucleolus of a pancreatic cell forms a substance prozymogen, which when it reacts unites with the elements in the cytoplasm to form zymogen. Hertwig (1902, 1903) and his pupil Goldschmidt (1904 and 1905) in their researches on certain Protozoa have pointed out that in abnormal states of the cell melanin-like granules are found in the cytoplasm and are due to nuclear discharges.

From our observations on the ganglion cells of the normal cat (Pl. XI, fig. 12) we are able to confirm Luzzani's work, and we regard the minute Negri-like bodies seen in this animal in the ganglion cells during normal health as due to a nucleolar discharge such as may occur normally in this animal.

At the same time we may draw attention to the fact that in our sections of the normal brain of this animal, we saw minute blood elements (blood platelets) staining similarly to Negri bodies and situated in the blood vessels: this fact will always have to be reckoned with in describing minute Negri bodies in the blood stream. From the above studies, we see that the nucleolar matter in certain circumstances may be wholly extruded into cytoplasm or may undergo fragmentation and that these fragments then migrate into the cytoplasm whilst the nucleolus itself returns to its original form. The fate of these nucleolar particles when in the cytoplasm differs widely. In some cases they may totally disappear; in other cases, they become swollen up, fused and modified into intracytoplasmic accumulations, so-called yolk-nuclei, or again they may blend with the cytoplasm and give rise to cytoplasmic structures (*e.g.* Negri bodies). Further it has been shown that when these nucleolar spherules are first extruded into the cytoplasm, and while they are still situated in the immediate vicinity of the nucleus, they take on a stain approximating to that of nucleolar matter from which they were derived. The further they become removed from the nucleus the more do they tend to lose their original nucleolar staining

reaction. We may conclude from these facts that nucleolar matter is not directly converted into specific structures (*i.e.* Negri bodies etc.) but only indirectly, by an interaction between it and the cytoplasm. This fact we consider has an important bearing on Negri body formation. We look on Negri bodies as the result of interaction between extruded nucleolar particles and the cytoplasm. This view will become more apparent from the following observations. We have found that usually the nucleolus of nerve cells in the rabies-brain instead of staining black by the iron haematoxylin and Van Gieson method stains a greenish colour in some animals and in some terracotta, indicating that some change has probably occurred to alter its original staining characteristics. With the methyl-blue eosin method of Mann it still however continues to stain the original red probably because this stain has a marked affinity for any chromatin structure even remotely derived from chromatin (*e.g.* the erythrocytes).

Instead of the nucleolus being always a single spherical body, there is frequently evidence that fragmentation is occurring in rabies-brains. In the rabbit this is most marked (Pl. XI, figs. 5, 6): and the nucleolus breaks up into a number of small particles (3 to 6) and these are then probably discharged into the cytoplasm in much the same way as in the case of the nucleolar discharges referred to above. These discharged fragments are in all probability the small Negri bodies seen in this animal and apparently do not increase in size with length of incubation. In the guinea-pig the nucleolus usually stains a greenish colour with the Van Gieson's mixture; it is generally irregular in shape and fragmentation may be seen. In this animal the Negri bodies also stain a greenish colour and are frequently cap-shaped, the base of the cap being hollowed out to receive the nucleus (Pl. XI, fig. 9). This appearance resembles the archoplasmic vesicle very closely, as seen in the sperm cell of this animal, and as we have said before, it was this fact which led us to undertake this investigation and connect the Negri body formation with nucleolar transformation. In the *dog* and *jackal* a few of the Negri bodies stain green, like the nucleolus in these animals, but the majority stain terracotta with Van Gieson's mixtures. Evidence of nucleolar fragmentation occurring in the ganglion cells of the hippocampus major of a dog is seen in Pl. XI, figs. 13, 14. We have only once seen this process so definite in the dog. The nucleolus as well as the particles outside still retain their iron stain, but the large Negri bodies (Pl. XI, fig. 13 *b'*) stain terracotta and show minute iron staining granules.

These small iron staining granules are regarded by some as being the actual parasites, round which the cytoplasm has reacted to produce the large Negri body. It is the cyst-like membrane which enshrouds the individual granules like a mantle that has led to their being named Chlamydozoa. The granules are not found in every case. In fact we observed them only in the dog and never in the guinea-pig or rabbit. Nor have we seen any evidence of a 'budding phenomenon' or dumb-bell-shaped division figures. These facts go in our opinion to support the view that the parasite of rabies has not yet been microscopically demonstrated. There is however no reason from our observations to negative the idea of the presence of a parasite as a cause of the phenomenon of Negri body formation. We next come to consider the significance of the yellow masses found in man, *i.e.* in the brains of subjects dying from rabies, and also seen to some extent in lower animals (*e.g.* mule and horse). These masses are apparently very constantly present in rabies, but they are not absolutely diagnostic. Can we consider them, on our hypothesis, to be in any way composed of substances derived from the nucleolus of the nerve cell? By Mann's stain the nerve cell containing these masses shows a nucleolus which exhibits a yellow centre with a red stained zone around it (Pl. XI, fig. 2), that is to say the centre of the nucleolus stains (or does not take a stain) in the same manner as the yellow masses in the cytoplasm. We saw that in certain ova (Text-figs. 1, 2; oöcyte of *Antedon bifida*) nucleolar discharges occur and give rise by aggregation to the so-called yolk nucleus[1]. These yellow masses, found in human brains, might easily be similar aggregations of nucleolar discharge set up by the action of a virus (like rabies virus) upon the nerve cell. From these researches and observations we are led to the following conclusions.

Conclusions.

(i) Negri bodies are not parasitic in nature, but are derived from extruded particles of nucleolar matter.

(ii) The extrusion is the result of katabolic changes in the nerve cells caused by the action of the rabies virus. Such an extrusion is also brought about by normal stimuli as is shown by the work of many observers.

[1] It may be advisable to avoid confusion by saying that the term here has no connection with the nucleus of a cell.

(iii) When the nucleolar particles leave the nucleus, an interaction occurs between them and the cytoplasm and so Negri bodies are formed.

(iv) The manner and the extent of the nucleolar discharge and the degree of cytoplasm reaction vary with different animals and thus the variations in structure and size of these bodies are accounted for.

(v) The iron staining granules in the Negri body are fragmented particles of nucleolar matter, which still retain the original iron staining character of the nucleolus.

(vi) Although these bodies are not truly specific for rabies yet their presence in marked numbers is very closely associated with this disease, probably in the same way as the Guarnieri bodies are for vaccinia (Ewing 1905) and the Plimmer's bodies for carcinomata (Borrel 1907, Farmer and others 1906, Greenough 1905). These latter bodies have already been regarded as being formed similarly to the structure known as the archoplasmic vesicle of a cell.

(vii) Thus we maintain that whatever the parasite for rabies may be it has not been demonstrated microscopically.

REFERENCES.

Babes, V. (1906). Les corpuscles de Negri et le parasite de la rage. *Presse Médicale*, xx.

—— (1907). Untersuchungen über die Negrischen Körper und ihre Beziehung zu dem Virus der Wutkrankheit. *Zeitschr. f. Hygiene*, lvi. 435.

Borrel, A. (1907). Le problème du Cancer. *Bull. Inst. Pasteur*, v. 497.

Calkins, G. N. (1910). *Protozoölogy*, p. 304, published by Baillière Tindall and Cox.

Chubb, G. C. (1906). The growth of the oöcyte in Antedon : a morphological study in cell metabolism. *Phil. Trans.*, B. 198, pp. 447–505, pls. 29–31.

Ewing (1905). The structure of vaccine bodies in isolated cells. *Journ. Med. Research*, viii. (New series) 233.

Farmer, Moore and Walker (1905). On "Plimmers bodies" and reproductive cells. *Pro. Roy. Soc.*, B. lxxvi. 230.

—— (1906). On the cytology of new growths. *Proc. Roy. Soc.*, B. lxxvii. 336.

Flemming (1882). *Zellsubstanz, Kern und Zellteilung.* Leipzig.

França, C. (1906). Diagnostic histologique de la rage. *Communic. au XVe Congrès de Lisbonne.*

Goldschmidt, R. (1904). Der Chromidialapparat lebhaft funktionirender Gewebezellen (Vorl. Mitt.). *Biol. Zentralbl.*, xxiv.

—— (1905). Die Chromidien der Protozoen. *Arch. f. Protistenkunde*, v. 126.

Greenough (1905). On the nature of cell inclusions in Cancer. *Journ. Med. Research*, viii. (New series) 137.

HARTMANN (1910). Ueber Chlamydozoen. *Centralbl. f. Bakteriol.* etc., Referate XLVII. Beiheft. p. 94.

HEIDENHAIM (1882). *Plasma und Zelle*, p. 212. Leipzig.

HERTWIG (1902). *Sitzungsber. d. Gesellsch. f. Morph. u. Physiol.*, XVII. 77.

—— (1903). Die Protozoen und die Zelltheorie. *Arch. f. Protistenkunde*, I. 1.

LUZZANI, L. (1905). Zur Diagnose der Tollwuth. *Zeitschr. f. Hygiene*, XLIX. 305.

MACALLUM (1891). *Trans. Can. Instit.* Toronto I. 247, ref. from Adami, J. G. *The Principles of Pathology*, Vol. I. p. 41, published by Hodder and Stoughton, London, 1909.

MONTGOMERY (1898). Comparative cytological studies with especial reference to the morphology of the nucleolus. *Journ. of Morphology*, XV. 2.

NEGRI, A. (1903). Beitrag zum Studium der Aetiologie der Tollwut. *Zeitschr. f. Hygiene*, XLIII. 507.

—— (1909). Über die Morphologie und den Entwicklungszyklus des Parasiten der Tollwut. *Ibid.* LXIII. pp. 421–443, pls. XV.–XVII.

PACE (1904). Sopra alcune formazioni eosinofile, simulanti i corpi di Negri etc. *Riforma Med.*, Ann. XX. No. 25, ref. *Baumgarten's Jahresbericht*, XX. 814.

POOR, D. W. (1906). The infectivity of tissues at different stages of hydrophobia. *Proc. New York Pathol. Soc.*, VI. 88.

STEINHAUS, J. (1890). Ueber parasitäre Einschlüsse in den Pankreaszellen der Amphibien. *Ziegler's Beiträge*, VII. 367.

WHEELER, W. M. (1896). The sexual phases of Myzostoma. *Mittheil. a. d. Zoologischen Station zu Neapel*, XII. 227, cited by Wilson (1906) q.v.

WILLIAMS, A. W. and LOWDEN, M. M. (1906). The aetiology and diagnosis of hydrophobia. *Journ. Infect. Diseases*, III. 452.

WILSON, E. B. (1906). *The Cell in Development and Inheritance*, p. 34, published by Macmillan & Co., New York.

EXPLANATION OF PLATE XI.

Except when otherwise stated Mann's methyl-blue eosin stain has been employed. The preparations were all drawn with the aid of a camera lucida, eyepiece No. 4, 1/12 apochromatic oil immersion, 11 mm. tube extension (Zeiss).

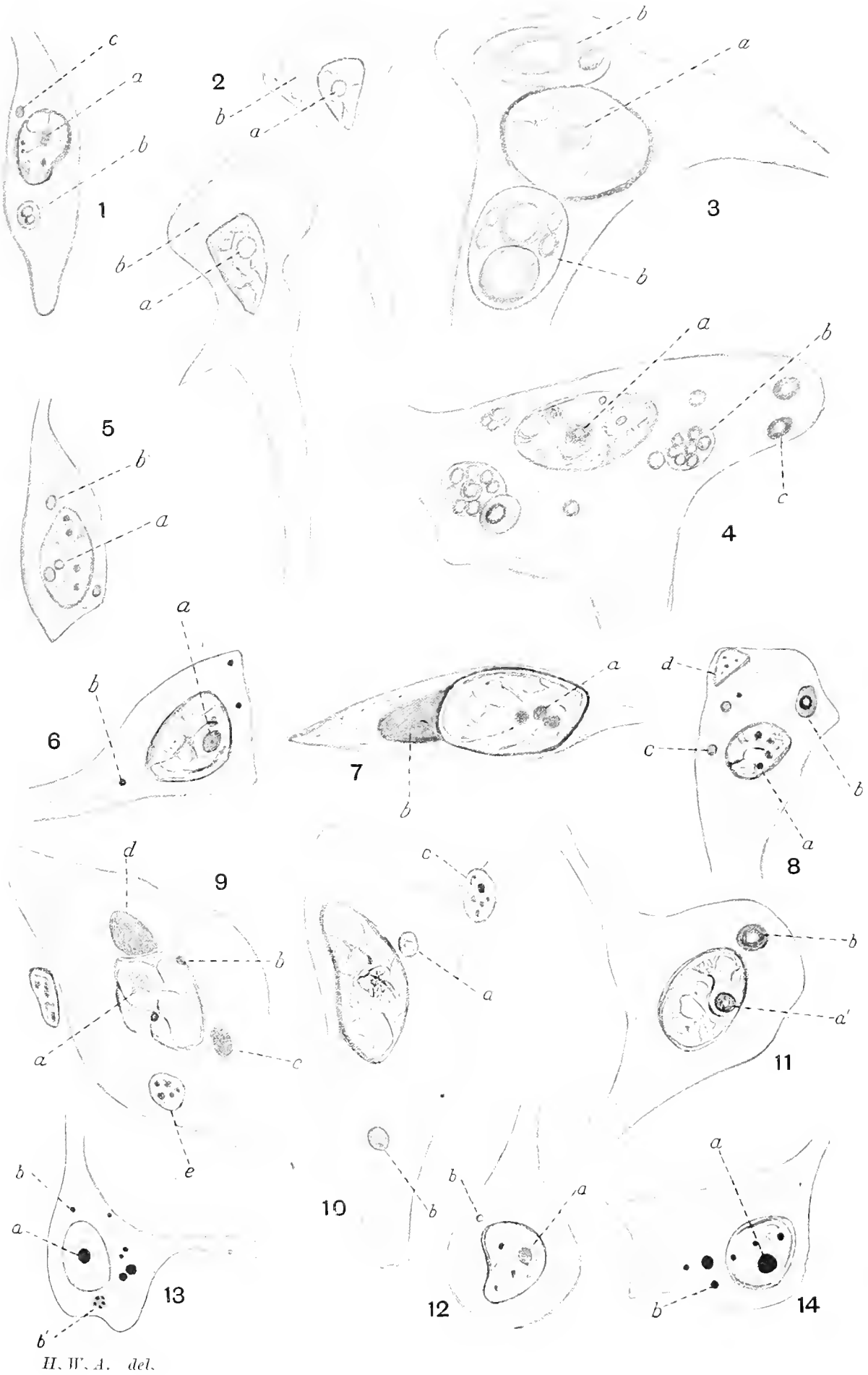
Note. In the authors' original crayon drawings, the cell protoplasm appears pink, the nucleus and neurogleal cells blue, the nucleoli and Negri bodies etc. red, except in figs. 13 and 14 where they are black. In fig. 2 the vesiculated mass (*b*) appears yellow and in figs. 10 and 11 the nucleoli (*a*, *a'*) appear green. ED.

Fig. 1. A cell from the fascia dentata of a guinea-pig killed with Russell's Viper venom, showing Negri bodies. (*a*) fragmented nucleolus; (*b*) vacuolated Negri body; (*c*) minute faintly staining cytoplasmic particle.

Fig. 2. Human cortical cells from a hydrophobia case, (*a*) nucleolus showing pink ring and yellow centre; (*b*) yellow vesiculated mass.

Fig. 3. Bullock infected with rabies; ganglion cell from the hippocampus major, (*a*) nucleolus; (*b*) large vacuolated Negri bodies with primary and secondary vesicles.

Fig. 4. Bullock ditto. (*a*) Nucleolus and fragmented particles; (*b*) and (*c*) large and small Negri bodies.



H. W. A. del.

Illustrating paper by Acton and Harvey.

Figs. 5 and 6. Rabbit, (5) Purkinje cell; (6) ganglion cell of hippocampus major. (*a*) Nucleolus and fragmented particles; (*b*) minute Negri bodies.

Fig. 7. Guinea-pig; cell from the fascia dentata. (*a*) fragmented nucleolus; (*b*) cap shaped Negri body.

Fig. 8. Guinea-pig Purkinje cell from cerebellum. (*a*) fragmented nucleolus; (*b*) large Negri body; (*c*) small Negri body; (*d*) neuroglial cells.

Fig. 9. Guinea-pig, ganglion cell of the hippocampus major. (*a*) irregularly shaped nucleolus; (*b*) nucleolar particle just being extruded; (*c*) large Negri body; (*d*) cap shaped Negri body; (*e*) neuroglial cell.

Figs. 10 and 11. Dog. Hippocampal ganglion cells. (Iron haematoxylin and Van Gieson.) (*a*) Extruded nucleolus stained green and vacuolated; (*a'*) green stained nucleolus; (*b*) Negri bodies; (*c*) neuroglial cell.

Fig. 12. Normal cat, ganglion cell of hippocampus major. (*a*) Nucleolus; (*b*) minute structure resembling a Negri body.

Figs. 13 and 14. Negri bodies of the dog. (Stained iron haematoxylin and Bordeaux red.) (*a*) Nucleolus; (*b*) small dark staining Negri bodies; (*b'*) large older Negri body (pink), showing the basophile granules of Volpino.

NOTE ON THE FOREGOING PAPER.

In the progress of our knowledge of the so-called Chlamydozoa, there have been four stages.

1. In the case of certain diseases, such as vaccinia and variola, scarlet fever, hydrophobia, trachoma, measles (?), foot-and-mouth disease (?), etc., inclusions have been found in epiblastic cells, epidermis or ganglion cells. These cell-inclusions have received names according to their discoverers, such as Guarnieri's bodies, Mallory's bodies, Negri's bodies, Prowazek's bodies, etc.

2. A certain number of investigators have taken the view that these cell-inclusions are the actual parasites; they have therefore given them generic and specific names such as *Cytoryctes vacciniae* and *variolae*, *Cyclasterium*, *Neuroryctes hydrophobiae*, etc. They have further described a supposed "life-cycle" for them, and have attempted to classify them in one or another group of the Protozoa.

3. Other investigators have come to the conclusion that these cell-inclusions are not parasites at all, and their life-cycles are purely illusory; they have shown that the cell-inclusions are degeneration-products of the cell-nucleus, consisting of extruded nucleolar substance.

4. Finally Prowazek, Hartmann and others have tried to prove that in these diseases true parasitic organisms are present in the form of very minute bodies which divide in a peculiar manner and are *quite*

distinct from the cell-inclusions in question. These organisms, by their pathogenic action on the cell, upset the relations of nucleus and cytoplasm and cause the nucleus to extrude nucleolar substance into the cell, thus producing the characteristic cell-inclusions. The parasites themselves become enveloped in these nuclear extrusions, as in a *mantle*, hence Prowazek proposed for these organisms the name Chlamydozoa.

The cell-inclusions must therefore be distinguished from the (alleged) true parasites; the names *Cytoryctes*, *Neuroryctes*, etc. must not be taken as denoting genera of Chlamydozoa. *Cytoryctes* and *Neuroryctes* are to be regarded as pseudo-parasites, consisting of masses of nucleolar substance extruded under the provocation of the true parasites, the minute, almost ultra-microscopic Chlamydozoa. Capt. Acton and Major Harvey do not seem to have grasped this difference, since on p. 256 they say "The term Chlamydozoa (Prowazek) is very generally used as a family name for this group of supposed organisms," *i.e.* for Guarnieri's bodies, etc., regarded as of parasitic nature. On the contrary, the "Chlamydozoa" theory is in direct antagonism to the view that Guarnieri's, Negri's, Mallory's, etc., bodies are of parasitic nature; it rests entirely on the view that they are nucleolar extrusions. This memoir, then, takes the question of hydrophobia to stage (3), and leaves stage (4) untouched.

ORIENTAL SORE IN BAGDAD, TOGETHER WITH OBSERVATIONS ON A GREGARINE IN *STEGOMYIA FASCIATA*, THE HAEMOGREGARINE OF DOGS AND THE FLAGELLATES OF HOUSE FLIES.

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(*The Report of the Expedition sent to Mesopotamia in* 1910 *by the London School of Tropical Medicine.*)

With Plates XII—XVI and 36 Text-figures.

CONTENTS.

Introduction.

THE present report is an account of observations made in Bagdad during the year 1910 (March to November) on the Oriental Sore as it occurs in this part of the world. Though the sore of Bagdad may not differ very markedly from that of other places, it is so common here that all stages and types of the sore may easily be studied, and such points as the early age of infection, immunity, and duration of the disease are very clearly exemplified. Accordingly, a more or less complete account of this disease of Bagdad, known locally as the Date boil or Arabic "Uchut," will be given, though descriptions of the sore as it occurs in several parts of the world have been published by other observers. During the investigations various side issues arose, and as these suggest certain interesting points, three sections have been added dealing with (1) the life history of *Lankesteria culicis* (Ross) a gregarine of *Stegomyia fasciata*, (2) *Haemogregarina canis* and its development both in the dog and the tick *Rhipicephalus sanguineus*, and (3) *Herpetomonas muscae domesticae*, and other flagellates of the house-fly.

During the first six months in Bagdad, I derived great help from my assistant Mr Stremes, but early in September there occurred a deplorable accident which resulted in the death of Mr Stremes, and the destruction of part of the laboratory by fire, together with the bulk of the

apparatus and outfit, and a large part of my collection of flies. No sooner was work resumed after the delay caused by this accident, than an epidemic of cholera completely disorganised my plans and put a stop to certain experiments I was about to conduct with the sand fly, *Phlebotamus*. Still, the results obtained as far as they go are of interest and will be found below in the following sections.

I. Oriental Sore in Bagdad.

Plate XIV.

A. *The disease as it occurs in man.*

As it occurs in Bagdad the disease is essentially one of childhood, and just as in England all children are expected to suffer from measles, so here the sore is looked upon as the natural portion of childhood. Being a disease which is accompanied by little or no constitutional disturbance, and in consequence by no death rate, it is not feared except for its inconvenience and the possibility of its leaving a disfiguring scar upon the face. The sore first makes its appearance as a rule, between the ages of one and three, and the face of the little child with one or more sores varying in size from a sixpenny piece to half a crown, over which are constantly feeding swarms of flies, is one of the commonest pictures of the Bagdad streets. Younger children may contract the disease, and I have seen the sore on the cheek of a baby seven months old,—but apparently there is little chance of the child becoming infected till it begins to run about and assert its independence in refusing to be completely covered up as is the manner of dealing with very young children in these countries. It occasionally happens that a child escapes infection and does not get the sore till later in life. Such cases are exceedingly rare, even if the evidence on which they are based is reliable. I have seen natives of Bagdad who have lived there all their lives, who declare that they never have suffered from the Bagdad sore. However, as the sore may be nothing more than a small papule of not greater diameter than five millimetres, and as it may never exceed this measurement, its presence could easily have been ignored. Though it is possible that a native of Bagdad may completely escape infection, it still remains a fact that the vast majority of the population contract the disease when young, and between the ages of one and three.

One constantly encounters cases of the sore in adults, but, with the very few possible exceptions just mentioned, these are in people who

have come to Bagdad from other districts in which the sore does not occur. Apparently the inhabitants of the smaller villages round Bagdad and the nomad Arabs living in encampments, are not so liable to the disease as those who dwell in the town. When these people come to Bagdad to seek employment, they contract the disease and furnish a good proportion of the adult cases met with. Europeans almost invariably become infected within a year or two after their arrival though in rare cases they may escape the disease for years, if not entirely.

All classes of the population are infected alike, and the child of the rich and well-to-do seems as liable to infection as the children brought up in the dirt and squalor of the most insanitary parts of the town. The children of the Europeans living in Bagdad generally suffer from the disease, and no matter what a person's nationality, a sojourn in Bagdad of a very few months or even weeks, is liable to result in a Bagdad sore which will leave its mark in the shape of the permanent scar, known locally as the "date mark." Such scars of varying size and extent are to be seen on the faces of the greater part of the population.

Seasonal prevalence of the disease. The sore may make its appearance at any time of the year, but it is a well recognised fact that the autumn, the time of the ripening of the dates, at the close of the long spell of hot weather, is the season when the majority of sores first develop. It is this fact which has given rise to the local name of "date boil" and "date mark" and to the hypothesis which attributes the sore to eating dates or even to injuries inflicted by the thorns of the date palms. The occurrence of the disease in early childhood does away with any such hypothesis. The month of September is the first month of the "date boil" season, and during this month and the two succeeding months, the majority of new cases occur. The frequency of onset at this particular season must certainly depend on some parallel variation in the occurrence of some biting fly or infecting agent responsible for its transmission. It is quite common for the sore to make its appearance at some other time of the year, but such cases may be instances of prolonged incubation period. It is evident that infection usually takes place during the hot months of June, July and August and with an incubation period of two to three months, the sore would appear, as it does, in the autumn. Cases are known where there is a much longer period of incubation, and the sores which develop at other seasons may be of this nature. It is possible that infection always takes place in

the summer months. This of course refers to the first inoculation, as secondary inoculations or autoinoculation may occur at any time of the year by the scratching of an already existing sore and the transference of some of the organisms to an abrasion on some other part of the body.

Position of the sore on the body. The strict limitation of the sore to exposed surfaces of the body has been a long recognised fact. This is one of the chief arguments in favour of insect transmission of the disease. In very young children less than twelve months old, one rarely encounters a sore except on the face, a fact probably dependent upon the habit the people have of wrapping infants up so that the whole body is covered with the exception of the face. At an earlier age the face is usually covered also, so that the rarity of sores in children before the age of twelve months is easily explained. In older children the sore commonly occurs on the arm or leg, below the elbow or knee, though the face is still the most usual situation. In adults, almost invariably new comers, the disease appears with about equal frequency upon the face, arms, or legs. It is rare to find a sore on any other than the exposed parts of the body. I have seen a case in a boy about five years old where there was one sore on the buttock, and a second over the sternal end of the second left rib, and in another child age about three years, a sore near the umbilicus. I have been told of cases where "sores" (?) occurred in numbers all over the body, but it must be doubtful if such are true sores (due to *Leishmania*) as the disease can very readily be confused with other conditions, such as syphilis.

Upon the face the sore appears usually upon the cheek or forehead. It commonly occurs on the end of the nose, producing an unsightly condition while it lasts though the permanent scarring is not in proportion to the temporary disfigurement. The sore more rarely attacks the lips, eyelids, and ears. I have not seen a case of sore on the scalp, neck, or on any of the mucous surfaces. On the arm the disease may appear on the elbow over the olecranon process, a position in which the sore is often very painful. More usually it is below the elbow and frequently occurs upon the fingers, producing a great enlargement of these. On the leg the favourite site is on the outer side just above the ankle joint.

This practically constant appearance of the sore on the exposed surfaces of the body can hardly be explained by any other assumption than that the disease is transmitted by some biting insect or fly which

feeds upon these exposed surfaces in contradistinction to such elusive insects as body lice, fleas, and bed bugs which generally suck their blood under the protection of clothing. In this connection however one must not forget the recent experiments of Basile on the flea transmission of canine leishmaniosis.

Number of sores on each individual. It is probably safe to say that the single sore is most commonly encountered, and this one may be on the cheek, lip, nose, eyelid, forehead, arm, hand, leg or foot. Two, three, and even four sores are by no means uncommon, but cases with larger numbers are certainly rare. It often happens that during the growth of a single sore secondary sores will develop on some exposed surface, either near the primary one, or further away. Such secondary sores are most likely subinoculations from the first. A child with a sore on its face will almost certainly inoculate any wound it may have upon its hand, and any slight abrasion of skin upon the face will be infected by the swarms of flies that are constantly walking across the sore. Some of these secondary sores may however be the result of a normal infection. I have seen the case of a child about 12–18 months old, where there were eighteen sores upon the face, one on each of the limbs except the left arm which had two, making a total of twenty-three sores. I have heard of cases where the numbers have been even greater, but the character of the sores in these cases had not been established by microscopical examination. In the case of this child with twenty-three sores, it is interesting to note that they were all limited to the exposed surfaces of the body. If the disease is the local manifestation of a general infection, it is difficult to explain how the sores limit themselves to the exposed surfaces only in these cases of multiple infections. One would expect that at least some would appear upon the body. On the other hand, it is not quite clear that each sore can be a separate inoculation by some fly, though in my opinion this is the more probable explanation.

Incubation period. On the subject of the incubation period it is difficult to obtain precise information from the observation of natural infections. The youngest child I have seen with a sore was seven months old. Cases of Europeans becoming infected within two months after reaching Bagdad, are not uncommon, and I have a reliable history of a case in which two red spots appeared upon the feet a fortnight after arrival. The subject of these sores was badly bitten upon the feet while sleeping on the roof without a mosquito net. All the marks produced by the bites disappeared except the two red spots mentioned.

These persisted for upwards of a year, and in them I was able to demonstrate the typical parasite. As a rule however the incubation period is longer and the sore may make its first appearance many months after a person has left the infected district. It is probable that the time elapsing between inoculation and the appearance of a noticeable sore is very variable and may depend on several factors, such as a person's natural resistance to the disease, the state of health at the time of inoculation, or the virulence and numbers of the parasite inoculated. From the first moment of inoculation the parasities must have been multiplying in the skin at the point of infection, and it is only when their number is sufficiently great that a visible change is produced on the surface.

From experimental inoculations more precise data can be obtained, though even here one must exercise caution in drawing conclusions, as the natural manner of infection may produce a sore more slowly or more quickly than by the inoculation of parasites from man to man. I inoculated a European lady on the left forearm with the juice squeezed from a sore on the arm of her husband. By microscopical examination, the juice was shown to contain numerous parasites. The inoculation was done by placing the juice on the skin and scarifying with a sharp instrument as in vaccination against smallpox. The wound caused by the scarification completely healed, though the mark or scar persisted and no definite development took place for some time. At the end of seven weeks from the time of inoculation, there was present at this spot a typical sore in the form of a small papule of about five millimetres in diameter. Later there appeared on the other arm two small sores which were probably the result of a natural infection if we remember Dr Saati's experiments. Though in this case the mark of the wound caused by the inoculation never completely disappeared, this is not usually the case as will be seen from the following interesting information on the subject of inoculation given me by Dr Saati of Mousul, who has practised protective inoculation for some time. He has been in the habit of inoculating people with the juice of the sore as in vaccination against smallpox, on the outer side of the leg. The sore thus produced heals quickly and finally all trace of the inoculation disappears. At the end of two months, or occasionally it may be three or four, there appears at the point inoculated a minute red papule which increases in size till it assumes the form of a typical sore. Dr Saati has inoculated about three dozen cases, and in the greater number of these the incubation period has been about two months. It is important to note that the

inoculation always produces only a single sore, and that at the site of the inoculation. It then may be accepted that the incubation period is approximately two months.

Character and development of the Bagdad sore. It is generally believed by the natives of Bagdad that there exist two kinds of sore which are designated male and female. Clinically this is true, for as the types of two extremes there exist the ulcerating and the non-ulcerating sore. The ulcerating as well as the non-ulcerating form of the disease commences as a minute red papule. This has a characteristic dusky red colour, which takes on a slightly brownish red tint, as it increases in size. It is only slightly raised at this stage, and does not produce any surrounding inflammation of the skin as does the common acne spot. In accordance with its chronic nature, it produces little or no irritation. It is not tender to the touch and would probably escape observation if it did not appear on a visible surface of the body. The original papule increases slowly in size and may finally follow one of two courses, which results in it being either a male or female sore. In the female sore the central portion breaks down leaving a shallow, indolent ulcer, from which exudes a yellow fluid in which are to be found the sore parasites and the numerous bacteria with which these ulcers become secondarily infected. This ulcer gradually extends till in severe cases a large area of skin may be involved. In one such case encountered the ulcer occupied the whole of one cheek, had destroyed the lower eyelid of this side, and extended across the bridge of the nose to the other cheek. Such extensive ulceration is however uncommon, the ulcers, as a rule, not exceeding the area of a five shilling piece. When small these ulcers frequently scab over with the dried up exudation. The scab is easily removed, and if allowed to remain, only serves to retain pus and make the ulcer painful by producing a surrounding inflammation. While extending in one direction the ulcers may heal at another part, producing a condition resembling that of a lupus. The surface of the ulcer is covered with granulations varying in colour according to the extent and nature of the secondary bacterial infection. The granulations readily bleed, and a scraping from the surface reveals a mixed infection of the typical parasite, bacteria and yeasts. One never obtains the specific parasite in such great numbers in these mixed infections. I have encountered no case of spirochaete infection in these ulcerating sores.

The second type of sore known as the male sore commences in the same manner as the female sore. The papule however increases in

size and does not ulcerate. The superficial layers may form into a dry scaly covering, which breaks away leaving a thin red skin beneath. If the scaly covering is forcibly removed, the skin may be broken showing a mass of red granulation tissue from which a pure culture of the parasite can be obtained. The granulations thus exposed soon scab over, and if left undisturbed the sore shows no tendency to further ulceration. Eventually the sore shrinks and becomes less elevated, till finally there is nothing left but a dusky red patch on the skin which gradually changes to a thin, white scar, very slightly depressed. The scarring left by the non-ulcerating male sores is never so extensive as that produced by the ulcerating variety in which the secondary bacterial infection has brought about a greater destruction of tissue. It must be remembered that the two types of sore just described are connected by intermediate forms, and a sore which has been progressing as a male non-ulcerating sore may suddenly break down and ulcerate. There is nothing in the character of the parasites to be obtained from the two types of sore that would lead one to suppose that there is any specific difference between them.

It is very rarely that any deformity results from the contractions of the superficial scars or date marks. In the case mentioned above in which there was extensive ulceration on the face, the lower eyelid was completely destroyed, so that considerable deformity would be bound to result. But such a case is very exceptional.

Duration of the disease. The duration of the disease is usually about one year with a variation of three months on either side of the limit. The advent of ulceration and the infection with extraneous organisms have little effect on the life of the individual sore. The male and female sores are of about equal duration.

Immunity. It is a general rule that after the sore has healed and disappeared the person is protected completely from further attack. I have been told of persons who have had a second and even a third attack with several years intervening between each. If such cases occur they must be extremely rare. The consensus of opinion amongst the inhabitants of Bagdad is that a second attack is impossible. The fact that sores in adults who have always lived in Bagdad are never seen, is a sufficient proof that the infection of childhood has conferred a life long immunity. As mentioned above, it is frequently the case that a second sore will commence during the course of the first. Such secondary sores may be simply an auto-infection. As it is possible to inoculate the parasite by simple scarification in the presence of juice from a sore, it

must certainly happen that a child will inoculate itself at some other place by conveying the organisms on its fingers. Such infection could also very readily be produced by the house-fly, though it has not been demonstrated experimentally. These secondary sores are liable to occur only because the immunity against the sore parasite is not complete. Sometimes the secondary sore will develop towards the closing period of the first. It is difficult to explain such cases on the theory of immunity. If it is an immunity which has caused the first sore to disappear, then one would expect this to be sufficient to prevent the development of the secondary sore. However, it is possible that the virulence of the parasites of the first sore has become so attenuated that they cease to multiply, while the freshly inoculated parasites of the secondary sore are of sufficient virulence to withstand the partial immunity which dissipated the first. I have the following history of the disease in a European: A sore appeared on the right wrist. This followed the usual course and disappeared in about one year. Soon after the disappearance of the first a second sore appeared on the left elbow. This persisted for a similar period, after which no more developed and the person has remained free since. In other cases a sore will develop up to a certain stage and then commence to shrink and show signs of disappearing, and just when the patient is congratulating himself that the trouble is nearly over, it will break out again, extend and exceed its original dimensions. With these exceptions, one single attack lasting about a year is the rule, while the healing of the sore appears to be brought about by an immunity against the specific organism acquired by the person infected; such an immunity being sufficiently lasting to protect the person from further attack for the rest of life.

Symptoms. Apart from purely local symptoms due to the sore, it is impossible to trace any constitutional disturbance. The fact that nearly all sores are in very young children renders the detection of such symptoms difficult, as any slight disturbance in health can at this age be attributed to the common ailments of childhood. The onset of the disease is so insidious that the first papule is not considered to be a true sore till its persistence compels this view to be taken. Mild fever, feelings of malaise or intestinal derangement, or other slight symptoms which might be supposed to accompany such a benign infection, would most probably be looked upon as one of those slight disturbances to health so common in all hot countries. In the case of the child with twenty-three distinct sores, recorded above, there was nothing to indicate, apart from the sores, that the child was in any way abnormal.

It has been suggested that this disease is a general one, and that a sore is merely the local manifestation of a general infection as in the case of syphilis. If this is true, there is however this difference; whereas in syphilis the chancre indicates a severe general infection accompanied by various definite symptoms, and a corresponding distribution of the specific parasite throughout the body, the sore is accompanied by no such constitutional disturbances, or such as are too slight to be detected, and the sore parasite is limited to the sore and does not extend to other parts of the body.

The local symptoms are due to the presence of the sore. In the early stages the papule may cause slight irritation and itching, and be feebly tender on pressure. There is only slight surrounding inflammation of the skin, except in some of the cases, with a secondary bacterial infection. There is little pain, and the sores are not remarkably tender unless it has formed over a bone such as the tibia or olecranon process. The discharge from the ulcerating sores is often very foul and exceedingly unpleasant, not only for others, but for the patient himself, who frequently is unable to remain in a room, but has to seek refuge in the open air. Occasionally, in septic sores, there is pain and tenderness in the lymphatic glands receiving the lymphatics from the infected area of skin.

The impossibility of performing autopsies on human beings in Bagdad is a misfortune, as from this source much light might be thrown on the disease. It is very important to know whether the parasites are to be found in the internal organs as in Kala azar, or whether they occur in the lymphatic glands. Without autopsies it is impossible to decide these questions. Repeated examinations of the peripheral blood of infected persons have failed to confirm the observations of Neumann made on a single case under his care in Heidelberg, that parasites occur occasionally in the circulation. Equally unsuccessful were the attempts to obtain cultures by inoculating tubes of blood agar with blood taken from the finger or ear of persons suffering from the sore.

Treatment. Very little can be done in the way of treatment and though many medicaments have been tried, they have no appreciable effect on the duration of the malady. Excision of the sore has been attempted, and with success. The sore must be treated as a malignant growth and removed entire with a good margin of healthy flesh, great care being taken to prevent contamination of the wound by the organisms of the sore. Such a procedure is hardly practicable on the face and would probably lead to more extensive scarring and deformity than if

the sore ran its natural course. One great disadvantage of such a treatment is, that to obtain good results the sore must be excised at a very early stage of its growth, and this would be before the immunity against the organism had been acquired and would leave the patient open to further infection.

Various ointments and lotions have a certain reputation amongst the inhabitants of Bagdad. The natives plaster the sores with strong solutions of indigo, or with the green alga which they scrape from the inside of the "hubs" the native earthenware filters. This continual application of medicaments is often continued till there is formed a large, dry crust over the sore which has the effect of shutting in the exudation and bringing about extensive suppuration. Judging from the difficulty of carrying on a culture of the sore parasite in blood agar in the presence of bacteria, it might be supposed that the infection of a sore with extraneous organisms leading to suppuration might have the desired effect of killing off the parasites. Though the suppurating sore does not disappear any more quickly than the non-ulcerating type, it is a fact that the specific parasites are to be obtained in much greater numbers from the latter. Dr Saati tells me that a saturated alcoholic solution of methylene blue has sometimes an advantageous effect. It does little to cut short the disease, but tends to keep the sore dry and free from ulceration. What can be the action of the methylene blue in these cases it is difficult to say. Very possibly the dryness is merely the hardening effect of the alcohol. The French authorities in N. Africa have obtained beneficial results by the dusting of the sore with permanganate of potash.

While in Bagdad I was in the habit of giving the yellow mercury ointment as an attractive medicament. It had no effect other than the cleansing of the sores, for I insisted that a careful washing of the sore was necessary before each fresh application. When once a sore has developed, the best course to adopt is to protect it from irritation, as this may start the unpleasant process of ulceration. It must be kept clean, and thus undisturbed, it will run the ordinary course for a year producing an immunity which will protect from further attacks.

Prevention. Unless the mode of infection is definitely determined, it is difficult to lay down rules for prevention. In all probability—and this is supported by the observations that the specific parasite will develop into flagellates in the gut of both the bed bug and a mosquito—the disease is conveyed from person to person by some insect which feeds upon exposed surfaces of the body. During the hot months of

the summer, it is the custom in Bagdad to sleep at night on the flat roof tops, and in the afternoons in the Sirdarbs, which are dark cellars partially below the ground. Both in the Sirdarbs and on the roof, people are liable to be bitten by all the biting flies found in Bagdad. An ordinary mosquito net will protect against any biting insects, but the *Phlebotamus*, which is able to pass through all but the finest mesh. In order to avoid infection it is essential to protect oneself against flies which feed upon one when asleep. The common house-fly is probably at least a mechanical carrier of the disease, and every care must be taken not to sleep in the day time without some protection against these insects which are constantly swarming over the exposed sores on the faces of the children. Any open wound on the face or hands is liable to be infected, and such should be carefully protected from the flies. In the case of children care must be taken to prevent them from inoculating themselves in other places by scratching the sores.

In a town like Bagdad where the disease is so common, though one may take every precaution it will be difficult to avoid the disease if one remains there any length of time. In such cases the most reasonable course to adopt is that of preventive inoculation. Such an inoculation will enable the person to choose the site of the sore and so avoid the inconvenience and risk of contracting the disease on the face with its resulting scar. The simple procedure of inoculation on the leg or arm would prevent the unsightly scars which one so commonly sees on the faces of the Bagdad inhabitants. Of course, in some cases sores would develop at other places than that chosen for inoculation, but such would be the result of a natural infection which had taken place at about the time of the inoculation. A protective inoculation of this kind would lessen very considerably the number of people having sores on exposed surfaces of the body.

This diminution in the number of sores on the exposed surfaces of the body, would in all probability have an important bearing on the spread of the disease. A sore on an exposed surface of the body, such as the face, is always an attraction to the myriads of flies which are to be found in localities like Bagdad. A sore on an unexposed surface would be free from these attacks and would not be a possible source of infection for others. With biting flies, such as mosquitoes, it is a little more difficult. If a biting fly is the agent of transmission, does this fly take up the parasite from the circulation or from the sore itself?

Though Neumann has found parasites in the peripheral blood on rare occasions, my repeated examinations in Bagdad have failed to

reveal these in the circulation. Attempts at culture from the peripheral blood have also failed. Hence it may reasonably be assumed that it is only rarely that the parasites are to be found in the general blood stream. There is nothing in the form or nature of a sore on the face, and especially in its early or non-ulcerating form, to make it repulsive to a mosquito. As will be shown later mosquitoes readily feed on the non-ulcerating sores, often preferring the thin red skin over these, to the tougher normal skin at the side. Mosquitoes feeding in this way may take up large numbers of parasites, and often, intact, the large mononuclear cells fully infected. Hence it is probably safe to conclude that if this disease is transmitted by a biting fly, this fly becomes infected by feeding from the sore directly, though much less frequently it may become infected by ingesting the scanty parasites from the peripheral circulation. It is thus very desirable to protect the sore against the attacks of the house-fly and any biting flies, and the simplest method of achieving this end is to develop the sore on some unexposed surface of the body by inoculation before infection takes place in the natural way. Protective inoculation in the way here advocated will not only protect the person from the visible deformity of a natural sore, but it will prevent him being a source of infection to others. By following out this plan on a large scale, it is not improbable that the disease could be very greatly diminished if not entirely stamped out. Till we know exactly how the sore is acquired, naturally such a line would be experimental, but it would be an experiment well worth carrying out and with every prospect of success. The inhabitants of Bagdad are accustomed to vaccination against smallpox and inoculation with sore virus has been done in an irregular manner, but generally on an exposed part of the leg. It would of course be necessary to inoculate on an unexposed surface. The promise that this would probably protect from a sore and scar on the face would in itself be a sufficient attraction.

Great care would of course have to be exercised in obtaining the virus from a reliable source without there being any chance of the conveyance of syphilis or tubercle at the same time. Should the cultures of the parasite in blood agar produce a sore which confers immunity, this would be the best source from which to obtain a virus. Recently Nicolle and Manceaux have been successful in inoculating the sore to men by employing artificial cultures, but we do not know if such sores will result in immunity against the natural infection. As pointed out above, inoculation with juice from a sore is readily effected by proceeding as in vaccination against smallpox. The usual course is the complete

disappearance of the scarification wound and the appearance of a sore in about two months at the point of inoculation. Inoculation at one spot does not produce sores at any other.

B. *Examination of the lower animals for signs of the disease and inoculation of these from man.*

Dogs. The commonest domestic animal brought into close association with man in Bagdad is the dog, which lives in a semi-wild condition in the streets and feeds on the refuse thrown out from the houses. Since in North Africa the dogs have been shown by C. Nicolle and C. Comte to harbour the parasite of infantile Kala azar, it was thought that dogs in Bagdad might in the same manner harbour the parasite of the Bagdad sore. There is moreover a general opinion that the dogs suffer from the sore about the nose or eyes. Cases of dogs with these supposed sores on the nose I had some difficulty in finding, as they were not nearly so common as they were said to be. Several cases were discovered, but unfortunately in none of these could the parasite be found. One large dog had a condition of the legs which was said to be the sore, but repeated examination of this failed to reveal the parasite. All the cases were in pet dogs living in private houses. The lesion on the noses of most of the dogs examined did not resemble the sore in man. They were more of a papillomatous nature and much harder to the touch than the true sore. They did not show signs of ulceration. In one small dog I saw a condition of the nose reminding one much more of the true sore. This was an ulcer scabbed over. The ulcer when I saw it had been in existence nine or ten months. Unfortunately, I was unable to examine this dog, which was also a pet in the house of an English resident.

Recently the authorities of Bagdad have followed the example of those of Constantinople in taking steps to exterminate the dogs from the streets. Accordingly, all street dogs have been collected and herded together in an enclosure in the desert where they are kept in a condition even less sanitary than that in which they lived before. Amongst these dogs there was a great mortality owing to lack of food, and I obtained permission from the authorities to perform autopsies on the dogs that died. I performed autopsies on a series of one hundred and ten dogs which I examined carefully for any signs of the sore about the nose or eyes. Where any ulceration occurred smears were made from scrapings of the sore. These smears together with portions

of the liver, spleen, and bone marrow of each dog, were taken back to the laboratory where they were examined microscopically for the typical parasite of Kala azar or the sore. In no instance was I successful in finding the Leishman-Donovan bodies, so that if the dog acts as an alternative host for the sore parasite, as it apparently does for the parasite of infantile Kala azar in North Africa and elsewhere, the percentage of infected dogs must be very low indeed and quite out of proportion to the number of cases of sore occurring in man. If the dog is an alternative host, one would expect to find the number of infected dogs approaching the number of infected human beings. The fact that no typical sore was found in any of these dogs throws no light on the question as to whether the Oriental sore is a purely local skin affection or a general infection representing a mild variety of Kala azar. At any rate, we can safely say that infections of this kind if they occur are very rare amongst the street dogs of Bagdad, a fact that does not lend support to the view that the dog acts as a reservoir for the parasite which is carried from them to men by some biting insect. The number of infected human beings is so great that they themselves act as centres of infection for others, and it is not necessary, in order to account for the incidence of the disease, to assume that the dog fulfils the rôle so often attributed to it. Though the dog may not act in this manner, it is possible that it may sometimes suffer from the sore in the ordinary way. However, such a case has not come under my notice.

By banishing the dogs from the streets, the Bagdad authorities have unknowingly carried out an interesting experiment. Assuming that the dogs act as a reservoir for the sore parasite, then their removal should bring about a great diminution in the number of human beings infected. The result of this experiment can be only apparent after some time.

The examination of this series of one hundred and ten dogs has shown that nearly every one is infected with the leucocytic haemogregarine first found by Bentley in India. The reproducing forms of this parasite were to be found in the spleen and bone marrow and in the ticks taken from the dog. The development of this parasite will form the subject of a later section of this report.

Piroplasmosis is common amongst the Bagdad dogs, which are generally in a very filthy condition and covered with fleas, ticks, and flies belonging to the group of the *Hippoboscidae*.

Inoculation of dogs. All attempts at inoculating dogs with the disease have failed. Dogs of all ages were used, from very young

puppies to full grown dogs. It was impossible for the young puppies to have suffered from the disease, so that the ordinary dogs of the Bagdad streets must have a natural resistance to the disease as inoculated experimentally. The inoculations were carried out in various ways. Some of the dogs were inoculated by scarification and application of fluid from a sore; others were inoculated into the skin with the hypodermic syringe. They were inoculated both on the skin of the nose, on the inner surface of the thigh, or on a shaved patch on the back. In none was a sore produced. In two dogs, in one case on the nose and in the other on the leg, a superficial ulceration was produced at the point of inoculation quite unlike a sore and in which the parasite could not be discovered, even after repeated examination. This failure to infect dogs with the disease is strange after the successful inoculation of dogs by C. Nicolle and L. Manceaux in North Africa. These observers have found that dogs become infected about the nose after an incubation period of thirty-six days. The disease however is not of long duration, so that it is possible that my failure to inoculate means that the Bagdad dogs are naturally immune in a place where the disease is so common in man. Inoculation of dogs from artificial culture of the sore parasite was equally unsuccessful.

Cats. Cats, like the dogs in Bagdad, though less numerous live in a semi-domestic condition. Only one of these animals was examined, and that with a negative result.

Rats. Rats were exceedingly common and a great pest. The only protozoal parasite found in the blood or organs was the common *Trypanosoma lewisi* and the leucocytic haemogregarine. Inoculation of numbers of rats with material from the sores and blood agar cultures has given no result.

Rabbits. Similar experiments conducted on rabbits have given no result.

Birds. Sparrows and two small owls were likewise inoculated without success.

Ticks. An attempt was made to obtain a development of the sore parasite by inoculating the large blown-out ticks taken off dogs. The ticks survived the operation of inoculation, which was made with a fine needle into the abdomen, but no trace of any developmental forms of the parasite could be found when the contents of the ticks were examined microscopically.

Other domestic animals in Bagdad are the horse, mule, donkey, and cattle. Camels are rarely seen in the town, and then only in passing

through from one gate to another. It is unlikely that any of these animals have an influenee on the disease as it oeeurs in Bagdad. It is popularly believed that not only dogs but other animals and even birds, sueh as eanaries, may suffer from the disease. It has beeome a custom to talk of any eurious and inexplieable skin lesion in any animal, as a sore. This is merely a superstition and is not supported by mieroscopieal examinations. In faet in human beings many skin eonditions are erroneously eonsidered to be the sore. This is espeeially true of a semi-ehronie ulcer from whieh white people eommonly suffer. It is easily distinguished however from the fact that it is more painful and tender, is surrounded by a greater area of inflammation and that it exudes a yellow pus. The organism of Oriental sore eannot be found in the scrapings, but a diploeoccus mueh resembling the gonococeus in appearanee is eonstantly present.

The failure to obtain an animal suitable for inoeulation experiments renders the investigation of the disease mueh more diffieult. If transmission experiments eould be earried out with biting flies as in the case of trypanosomiasis, one eould more readily arrive at results. In the case of the sore all sueh experiments have to be earried out on man, and it is diffieult to find subjeets suitable and willing. In want of these experiments the following eourse was adopted. Flies of various kinds were fed upon the sore and these were dissected after varying intervals and search made for developmental forms of the parasite, a proeedure whieh is much more difficult and full of pitfalls than the experiment of transmission. In the seareh for developmental forms one is guided by the changes in form undergone by the parasites during their evolutions in the blood agar eulture.

C. *Examination and dissection of flies and ticks.*

There are of course in Bagdad a large number of arthropods any of whieh ean be looked upon as possible transmitters of the parasite of the sore. In order to obtain some idea of the intestinal fauna of these arthropods, a dissection of such as could be eaught was undertaken. This was a necessary preliminary to any feeding experiments that were to be made.

House-flies. These oeeur in great numbers as in all oriental towns. They appeared to diminish in numbers to some extent during the hottest part of the summer when the maximum shade temperature reaehed 110° F. The great heat eombined with the dryness of the

atmosphere seems to have a deleterious effect upon them. When the temperature is not so high the flies abound and are constantly swarming about the faces of the children and more especially those made attractive by the sore. Such flies collected from the face of a child suffering from an ulcerating type of sore are found to have the intestine filled with the exudation of the sore in which the parasites can readily be found. This was only to be expected, as any fly feeding on the sore is bound to take up large numbers of parasites. Such a fly feeding immediately afterwards upon some fresh abrasion of the skin must certainly in a number of instances inoculate the sore parasite.

On carrying out the dissection of house-flies, one was not surprised to find that a certain percentage of these had an intestinal infection of *Herpetomonas*. In some instances these appeared to be *Herpetomonas muscae domesticae*; in others there occurred a smaller flagellate, also of the *Herpetomonas* type. In a certain number of flies the malpighian tubes were infected with flagellates which were of the trypanosome type with the kinetonucleus on the non-flagellar side of nucleus. A description of these flagellates will be found in another section. Whether these various flagellates represent different stages of one parasite of the fly is a point difficult to settle, unless one undertakes special experimental work to this end. The smaller forms of herpetomones from their resemblance to the cultural forms of the Leishman-Donovan bodies might readily be taken for similar developmental forms in the gut of the fly. Judging from experiments conducted with house-flies it is improbable that the *Herpetomonas* are related to *Leishmania*. The intestine of the fly was filled with all kinds of débris intermingled with bacteria of many kinds.

Stomoxys. From the time of my arrival in Bagdad in March till the hot weather had set in in June, these flies occurred in fair numbers, especially in the neighbourhood of the stables. They often settled upon one and were mistaken for house-flies till the pricking of the proboscis revealed their nature. They would frequently bite about the ankles, especially through dark socks. With the advent of the really hot weather these flies almost completely disappeared and could rarely be discovered even in the stables, their favourite haunt. The laboratory where these investigations were conducted was situated directly opposite a stable, and here during the cooler months it was an easy matter to capture forty or fifty of these flies in a very short time. During the hot months of July, August and September, a whole morning's search would fail to yield a single specimen.

These flies feed naturally upon the horses, donkeys, and mules, so that blood from these animals is found in their intestines. *Filaria* larvae were often encountered, and occasionally in the hind gut a flagellate of the *Herpetomonas* type. *Stomoxys* occasionally was caught feeding on the faces of children with sores, but in no instance were the parasites found in the intestine of such flies.

Hippoboscidae. Flies belonging to this group are to be found commonly on the horses. Another species is constantly present on the dogs, where it lies concealed in the fur till disturbed, when it will leave its host, or fly to hide on some other part of the body. These dog flies will bite human beings, but this is an uncommon occurrence. I was bitten only on two occasions though I lived in close association with a number of dogs, all of which had many of these flies about them. Numbers of the flies were dissected from time to time, but none of these were found to be infected with flagellates.

Tabanidae. Flies belonging to this group I have not encountered in Bagdad, but I have seen one taken a mile or two out of the town.

Fleas. Fleas are a great pest, and especially at a certain season of the year which is popularly known as the flea season. They occur in greatest numbers in the month or two before the commencement of the summer season of intense heat. They are constantly on one's person during these months and are a continual worry to anyone who is susceptible to the irritating substances they inject. Dissection of fleas has not revealed any flagellate infection. If fleas are responsible for the transmission of the sores as Basile finds they are for canine leishmaniosis then with an incubation period of two months the greatest number of sores should appear in June or July. This is not so however as the greatest number of sores appear in the autumn.

Body lice. These are found commonly on the bodies and clothes of the dirty portion of the Bagdad population, and many were dissected without result.

Ticks. Ticks are found on practically all the domestic animals. They however rarely attack man. The dogs in the streets are usually covered with them. Dissections of these ticks taken from dogs have disclosed no flagellates but on several occasions a larval *Filaria*, probably *Filaria immitis*, was found in the gut and frequently the developmental stages of the leucocytic haemogregarine of the dog, a description of which will be found below in another section.

Bed Bugs. These occur in Bagdad, but in no great numbers. They can usually be obtained fairly early in the prison where they hide in

the mats covering the floors or upon which the prisoners sleep. In only one other house have I heard of the occurrence of bed bugs. It is certain that Bagdad is not infested with bed bugs to anything like the extent of some other Eastern cities. This statement is borne out by the evidence of the European residents, who would readily detect the presence of these pests even if they passed unnoticed by the native; Europeans generally believe that the bed bug does not exist in Bagdad. It was only by instituting a careful search and enquiry that I discovered their whereabouts in the prison. It is possible that the dryness of the atmosphere combined with the intense heat of summer is unfavourable to their extensive development and spread.

Owing to the incriminating evidence brought against the bed bug by Patton in the demonstration that the parasite of Kala azar develops into *Herpetomonas* in its gut, this insect was looked upon with suspicion and was made the subject of a careful enquiry. Firstly, numbers of bed bugs were dissected as they were taken from the prison. Many of them had recently fed upon human blood, while others had not fed for varying intervals judging from the condition of the intestinal contents. In this way seventy-two bugs were dissected, smears were made of the intestinal contents and examined microscopically for flagellates. In none was I successful in finding any protozoa. This result is important in the light of the results of the experiments to be detailed below.

Sand flies. This name is used in Bagdad for any small fly that bites at night. The fly most usually described under this name is a *Phlebotamus.* It is able to pass through the meshes of the ordinary mosquito net from which it may or may not escape after feeding in the morning. To protect oneself against the bites of these insects, it is necessary to use fine meshed nets. Unfortunately, when I wished to commence experiments with these flies, very few could be obtained, so that no definite result was arrived at. This is to be deplored, for it is possible that this fly may be the transmitting agent of the disease. The delay in these experiments was caused by the unfortunate fire that took place in my laboratory, and by the outbreak of cholera which put a stop to a scheme I had planned for continuing the work. However, I hope to be able to continue these experiments at a future date.

Mosquitoes. These occur, as would be expected, in large numbers. In some years the Tigris overflows its banks and runs into the desert around Bagdad, forming pools and marshes. Under these conditions mosquitoes are said to be much more numerous than I have seen. The

mosquito fauna of Bagdad differs very much from that of Busra, which is situated near the mouth of the river. Here anophelines are very common, and in consequence there is much malaria. In Bagdad on the other hand, I did not see a single specimen of an anopheline or malaria carrier, and all the cases of malaria that I met with had come from Busra or some other town in which malaria occurred. I was told on good authority that anophelines may occur in Bagdad and that cases of malaria contracted there are sometimes encountered.

Before the hot weather had commenced from March to June, various species of *Culex* were common. Amongst these was the common *Culex fatigans.* With the advent of the hot weather the number of *Culex* apparently diminished, while another mosquito, which I had not met before, began to make its appearance. This was *Stegomyia fasciata.* As the summer advanced the numbers of this voracious insect increased till it became a constant nuisance. It lives in the houses and hides in any dark corner, especially in the Sirdarbs or semi-underground rooms into which one retires during the hottest part of the day. It is most persistent in its attacks so that one is unable to escape from its ravages unless one is protected by a mosquito net. Both the male and female are fond of alighting on the skin, but though the male apparently makes attempts to perforate the epidermis by probing about with its proboscis, it is only rarely that it gains any satisfaction in this way. It seems as if the male is attached to the human being for other motives, for repeatedly I have watched several males attempting to bite without success. The approach of a female has diverted the attention from these fruitless efforts, and the males have attacked the female on the wing and at least one and sometimes two at one time, have been successful in attaching themselves to a single female. The males seem to hover around the human being not so much to obtain a feed of blood, as because they know that before very long a female will approach.

The females, on the other hand, feed very readily, and twenty-four hours is often sufficient time for the digestion of the large quantity of blood taken up at a single feed. The female will readily feed every day.

The breeding places of the mosquitoes are generally the wells with which most houses are supplied, the large porous earthenware water filters known locally as "hubs," or the cesspools which are generally under the courtyard of each house. The cesspool communicates with the exterior through a small hole over which a round stone is rolled. Some of the wells in disused houses become very foul, and from them

enormous numbers of *Culex* come forth daily. The *Stegomyia* breeds very readily in the cleaner wells and also in the "hubs" which are filled daily with water carried from the river in skins. If left for more than a week without being emptied and cleaned, the hubs become the source of numbers of *Culex* and *Stegomyia*. During the hot weather the development is very rapid, and one week is almost sufficient for the complete development of the mosquito from the egg. During the very dry and hot season the mosquitoes rest during the day upon the moist outer surfaces of these hubs, the temperature of which is considerably below that of the surrounding objects. In the summer the air is very dry, and though a good deal of irrigation of gardens takes place, the water carried up each day from the river has dried up and evaporated before night, so that there is little chance of mosquitoes breeding in standing pools. In the town, by cleaning out the hubs regularly, covering over used or old disused wells, and by attending to the cesspools, it would be possible to rid the town of most of its mosquitoes. It is true that the river Tigris runs through the town. During the summer months the climatic conditions resemble very much those of Khartoum on the Blue Nile, and there under very similar conditions, Dr Balfour has found it possible to exterminate the mosquito. Of course, it would be impossible to carry out such anti-mosquito measures in an old town like Bagdad, for much of it is in a very insanitary condition, and the carelessness of the Eastern native would be a constant obstacle. Khartoum is a comparatively new town in which inspection and enforcement of regulations are not a very difficult matter. But Bagdad is an old town with narrow crowded streets, and many small and dirty houses in which the poorer part of the population are crowded together in conditions far from sanitary. To enforce regulations among these people is almost impossible. However, it would be possible to educate the more enlightened part of the population, and this at any rate would have the effect of diminishing the mosquitoes in the town, and thus one of the possible agents of transmission of the sore. As a matter of fact, during my stay in Bagdad the newly appointed progressive Wali moved in this direction by causing many old and disused wells to be filled in and completely covered over and by building a dyke across the bend of the river on which Bagdad stands in order to prevent the flooding of the desert around the town when the Tigris overflows its banks.

Dissection of mosquitoes as caught in and about the houses was constantly carried on. On no occasion was a flagellate found in these

wild mosquitoes. In *Stegomyia fasciata* a gregarine first noted by Ross in India, was found in the encysted condition. This gregarine was also found in the larvae and pupae, and a description of its life history will form the subject of a later section of this report.

In the larvae of *Stegomyia fasciata* a flagellate (*Herpetomonas*) was occasionally encountered both in the gut and malpighian tubes. It was never met with in the pupa nor in the adult mosquito. The presence of this flagellate in the larvae is of importance from the point of view of the experiments made by feeding *Stegomyia fasciata* on the sore. The larvae very commonly have a large spirochaete infection of the intestine.

Reduvidae. On two occasions only did I encounter members of this group. Only two examples were seen, and these were both very small forms which had alighted on my hand. One definitely bit my hand. It was captured and mounted. The presence of a member of this group is interesting, as a suggestion has been put forward by Donovan that possibly some reduvid may be responsible for the transmission of Kala azar and we know that the *Schizotrypanum* in South America is transmitted in this way. The numbers of *Reduvidae* in Bagdad are however too small to be able to account for such a common disease as the sore.

D. *Experiments undertaken with the object of infecting flies with the parasite of the sore.*

House flies. As already mentioned the house-fly is most persistent in its attempts to feed on the sore, and of all flies which might possibly feed from the sore, the house-fly certainly takes up more parasites than any other. On this account the house-fly has been looked upon with suspicion, and Dr Row has suggested that the house-fly is the natural agent of transmission of the disease. There can be no doubt that the house-fly occasionally carries the parasites to open wounds, and in this way produces sores, but it is unlikely that every sore is the inoculation of an abrasion of the skin by a house-fly, and more improbable still that the house-fly can inoculate the individual through healthy skin. I have said that, as was to be expected, house-flies collected from the faces of the infected children show numbers of the sore parasites in the gut. The same result is obtained by allowing the house-fly to feed on the open sore or on juice or scrapings from the sore. In order to determine if any development would take place in the

house-flies, they were fed on the sore one or more times and dissected at varying intervals after feeding. The possibility of the occurrence of a natural flagellate infection has to be remembered in these experiments, which were all made with flies caught about the house.

1. In every fly dissected immediately after feeding on the sore or on the exudate or scrapings from this, Leishman-Donovan bodies were found.

2. Flies fed in the same manner and dissected five hours after feeding gave negative results. The parasites had disappeared. In some of these flies natural flagellates were found, but there was no possibility of mistaking these for the sore parasites. The fact that natural flagellates occurred in a few, does not affect the result which was the disappearance of the parasites in such a short time in the large number of flies employed.

3. Batches of flies were fed on the sore daily, and a certain number dissected each day. In every case twenty-four hours had elapsed since the last feed when the dissection was made. In this way flies dissected had had one to ten feeds from the sore. Those that had had ten feeds must have taken up enormous numbers of parasites, but in spite of this no evidence could be obtained that any development had taken place. The maximum number of feeds any single fly had was ten, and the dissection was made eleven days after the first feed. The repeated occurrence of the natural flagellates in the experimental flies might tend to obscure the result, but the proportion of infected flies amongst those that had fed on the sore, was not greater than those fed in other ways. To test this latter point control flies were fed daily on human blood from an uninfected person. The different batches of flies were not all kept at the same temperature. Some were kept in the laboratory where the temperature was high and frequently attained 80°–110° F. for the twenty-four hours. Others were kept in the Sirdarbs where the temperature did not rise much above 80° F., and others were kept in small porous earthenware jars covered with mosquito netting, and standing in plates of water. The water soaked up the sides of the porous pots, and by evaporation the temperature was considerably lowered and registered inside the pot from 70° to 75° F. In all cases the results were the same and no evidence of development was discovered.

In some series of flies it was found that the number of individuals with a flagellate infection was greater amongst those that had not fed on the sore. For instance, twenty flies were fed daily for ten days on

scrapings of the sore, and another twenty daily on citrated human blood, for the same length of time. The surviving flies (twelve and fourteen respectively) were dissected twenty-four hours after the last feed. In those that had fed upon the scrapings of the sore no trace of *Herpetomonas* could be found, while in two of the control flies flagellates probably *H. muscae domesticae* occurred. In such an experiment the natural fly infections did not affect the result. In other cases the findings were reversed, but in none was there any evidence of a development of the specific parasite of the sore.

Examination of the faeces of infected flies was always negative. In carrying out these experiments the gut was removed to a slide and films made from the contents taken from the stomach and intestine. These films were stained with Giemsa's stain and examined for parasites.

The constant presence of large numbers of bacteria of various kinds in the intestine of the house-fly may account for the quick disappearance of the parasite when taken up. As in artificial cultures the sore parasites do not develop, or only to a limited extent in the presence of bacteria, so one is not surprised to find that no development takes place in the gut of the house-fly.

As will be shown below in the artificial cultures in rabbits' blood agar, the *Herpetomonas* resulting from the sore parasites may be extremely minute and merely little flagellate organisms of not more than 2 μ in diameter. Such minute forms would be extremely difficult to detect amongst the mixture of substances and bacteria one finds in the gut of such an omnivorous feeder as the house-fly. However, one would at least expect to find some larger forms as in the artificial cultures. House-flies were also allowed to feed on cultures of the sore parasites in rabbits' blood agar. Results obtained were similar to those obtained by feeding the flies on the sore. The flagellates taken up very quickly degenerated and disappeared.

Stomoxys. Numerous experiments were made with these flies which were caught for the purpose in the stable near the house. The flies occurred in greatest numbers at the early part of the summer. During the hottest season they were difficult to secure. The flies taken in the stable had in most cases already had a feed of blood from the horses, so they were starved for twenty-four hours after which time they readily fed on the sore. It was not so easy to discover the parasites of the sore in the stomachs of *Stomoxys*, even when dissected immediately after feeding as in the case of the house-fly. The difference in the method

of feeding in the two cases would account for this. It was impossible to determine what percentage of *Stomoxys* took up parasites from the sore without feeding very large numbers on the sore, and dissecting immediately. However, in some cases large numbers of parasites were taken up and even the large mononuclear cells full of parasites, and a short examination of the stomach contents sufficed to demonstrate their presence, so that it is possible that in those cases where the parasites were not found they were present in small numbers only. The experiments with *Stomoxys* were conducted on the same lines as those made with house-flies, with the difference that the *Stomoxys* were not fed on scrapings from the sore. The greatest number of feeds given a *Stomoxys* was ten, and the flies were dissected twenty-four hours after the last feed. This time generally sufficed for the complete disappearance of the blood taken up twenty-four hours before. As in the case of the house-flies the parasites very quickly disappeared from the gut and on no occasion was any trace of a development discovered. The presence of a *Herpetomonas* in the *Stomoxys* tended to obscure the result, but the flagellate was only found once in a fly fed on the sore, though several times in flies dissected immediately after capture in the stable. The *Herpetomonas* is either a flagellate peculiar to the fly, or represents some trypanosome of the horse or other animal.

Dog Flies (Hippoboscidae). I was not successful is inducing these flies to feed on the sore, and they very quickly died if kept in confinement away from a dog.

Fleas and Pediculi. Experiments with fleas and body lice were not made though numbers of these were dissected without result.

In view of the experiments on the transmission of canine leishmaniosis recently recorded by Basile, the possibility of fleas transmitting the sore must not be forgotten.

Canine leishmaniosis of this type is however a general infection, while the sore is a local skin disease. In the latter one can safely assume that the sore develops at the site of the inoculation whether this be carried out naturally or artificially. In such a case the flea can hardly be responsible for a lesion appearing exclusively upon exposed surfaces of the body.

Bed Bugs. Experiments were carried out by allowing the bed bugs obtained from the prison to feed on the sore. Only a small percentage of bugs would feed under these circumstances, even after prolonged starvation, so that it was a laborious process inducing the bugs to feed. When they did feed they became gorged with blood. Bugs which had

fed were dissected at varying intervals after feeding. On no occasion was I successful in inducing a bug to feed twice from the sore. In all, twelve adult bugs fed on the sore, and became gorged with blood. Two of these were dissected twenty-four hours after feeding, eight were dissected forty-eight hours after feeding, and the remaining two seventy-two hours after. Developmental forms of the sore parasites were found in the stomach of one which was dissected twenty-four hours after feeding, in three which had fed forty-eight hours before, and in neither of those dissected seventy-two hours after. So that in four out of the total of twelve fed on the sore were flagellates found.

Four young bugs which had hatched from eggs laid by bugs in the laboratory fed on the sore. Only three of these were dissected, all with a negative result.

It has been mentioned above that seventy-two bugs taken from the prison and dissected immediately, gave no indication of a flagellate infection, so that there seems little doubt that the flagellates found in the gut of those fed on the sore represent cultural forms of the sore parasite. It is unfortunate that it was not found possible to conduct a greater number of experiments with the bugs hatched in the laboratory, but the greatest difficulty was experienced in persuading the young bugs to feed on the sore. The great majority of the many tried either persistently walked away or became involved in the exudate and there perished.

The flagellate developmental forms of the sore parasite in the bed bugs are figured at Pl. XII, figs. 1–21. It will be seen that they very closely resemble the cultural forms obtained on blood agar. Forty-eight hours after having been taken up, all are in one flagellate condition, while in the bug dissected twenty-four hours after feeding, rounded forms and clumps of incompletely developed parasites are found in addition to completely developed flagellates. In the bugs many abnormal forms are encountered, the protoplasm is often vacuolated and the nucleus broken up or absent. It seems probable that the development in the bug is an abortive one and takes place on account of the large quantity of blood which has acted as a good culture medium.

These observations are very similar to those made by Patton on the development of the parasite of Kala azar in *Cimex rotundatum*. If the parasite of the sore can develop into a flagellate in the stomach of an insect, not its true host, then it would be expected that the very similar parasite of Kala azar would develop in the same manner. Such experiments and results only show that it is unsafe to draw the conclusion

that an insect showing such developmental stages in its gut is the true carrier of the disease, for there is no question of the possibility of the bed bug being the carrier of the sore in Bagdad. This development is then merely a partial imitation of what would actually take place in the true intermediate host.

Mosquitoes. During the earlier part of my stay in Bagdad and before the commencement of the hottest season, various species of *Culex* (including *C. fatigans*) were very common, and numbers of experiments were made with these. It was exceedingly difficult to induce them to feed upon the sore and it was only rarely that one would feed more than twice. In all thirty-one mosquitoes other than *Stegomyia fasciata* chiefly *C. fatigans*, were fed on the sore. Some of these fed twice and one three times, but the majority fed but once. These were dissected twenty-four, forty-eight and seventy-two hours after feeding. No trace of the sore parasites or developmental forms could be discovered. Five mosquitoes were induced to take up exudate from the sore but the parasites could not be traced.

Owing to the difficulty of inducing these mosquitoes to feed on the sore, the experiment was tried of turning loose mosquitoes into a net under which a boy with a sore on his face was sleeping. Those mosquitoes which had fed were collected in the morning and dissected after varying intervals. Of course in such an experiment only a small percentage of mosquitoes would have fed on the sore and taken up parasites, unless we assume that parasites are taken up from the peripheral blood in support of which assumption we have seen above there is little evidence. Close on one hundred mosquitoes were examined after feeding in this manner, but with negative results.

Much difficulty was experienced in keeping the *Culex* alive in captivity, as frequently whole batches would be found dead though they had appeared healthy a short time before. In these cases it was found that the intestine and even the other organs of the body were teeming with a bacillus which had evidently destroyed them.

With *Stegomyia fasciata* which first began to make its appearance in June, experimental work was much more readily carried out. These mosquitoes feed greedily in broad daylight, and are quite willing to feed from the sore every twenty-four hours. It was easy to make them feed on any spot on the sore by gently guiding the proboscis to this spot. So eager were they to feed that they were not unduly disturbed by this interruption. While feeding the proboscis was generally plunged in to its base, and if the blood did not flow readily, the

proboscis was partly withdrawn and again inserted till the mosquito was satisfied with the supply of blood it was drawing. These mosquitoes more often prefer to feed on the thin red skin at the margin of the sore than on the healthy skin beyond. The rapidity with which the relatively enormous quantity of blood taken up at a single feed is digested and got rid of, is remarkable. In twenty-four hours the blown out *Stegomyia* will have returned to its normal size and be ready for another feed.

Stegomyia fed upon the sore and dissected immediately afterwards were found to have taken up the parasites in about 10 % of cases. In the others the parasites may have been too few for detection. In no instance were flagellates found in *Stegomyia* which had had only one feed on the sore. In one *Stegomyia* which had fed on the sore on two successive days and was dissected twenty-four hours after the last feed, there were found rounded forms of the parasite which resembled the enlarged forms seen in the early stages of the artificial cultures.

In five other *Stegomyia* which had had a number of feeds varying from four to ten, and which had been dissected either twenty-four or forty-eight hours after the last feed, fully formed flagellates were found (Pl. XII).

In all, over eighty *Stegomyia* were fed in this way, the majority of these having had over five feeds and many of them ten. So that out of the eighty *Stegomyia* fed on the sore six were found to have evidence of a flagellate infection of the gut. This is a much smaller percentage than in the case of the bed bug, and here one can be less certain that one is dealing with developmental forms of the sore parasite and not with natural flagellates of the mosquito. In each case the hind gut was free from flagellates, a fact which is in favour of their being derived from the sore, since in natural infections the hind gut is generally the seat of the most intense infection. The experiments in nearly every instance were conducted with mosquitoes which had their first feed on the sore.

The result is complicated by the presence of a *Herpetomonas* occasionally in the gut and malpighian tubes of the larvae. On no occasion however have these flagellates been found in the gut or malpighian tubes of the pupae, nor in the mosquitoes which had not fed on the sore though very many were dissected.

As controls to the feeding experiments some six dozen *Stegomyia* were allowed to have one or more feeds on a human being, but in none of these were any *Herpetomonas* found. So that the evidence is in favour

of the flagellates found in *Stegomyia* being a cultural form of the sore parasite as in the case of the bed bug.

Attempts were made to infect mosquitoes by allowing them to feed on the artificial culture, but with no definite result. The flagellates so taken up, quickly disappeared from the gut, even though the mosquitoes were allowed to feed on a human being afterwards.

The experiments with the flies just recorded were conducted mostly on a boy of about four years of age, who had a sore of the non-ulcerating type on the cheek. Some were made with other patients, but the boy was the only one who was willing to be employed in this way regularly. One had to be very careful not to frighten the patient or his friends, who were always very suspicious of what was being done. The sore was a non-ulcerating one, and in order to allow house-flies to feed from it, it was necessary to remove some of the thin skin over it. This exposed the red granulations from which it was easy to obtain large numbers of parasites. House-flies feeding on these granulations took up numbers of the large infected mononuclears and also many free parasites. A scab formed from day to day over these granulations, and this was removed whenever it was necessary to feed house-flies or to obtain juice from the sore.

With mosquitoes and *Stomoxys* it was not necessary to have the scab removed, as these insects fed readily through the intact skin at the side of the scab. Bed bugs generally would not feed, unless the granulations were exposed, and this introduced a difficulty, for immediately the bug became in the least involved in the exudate, it refused to feed, and often died, especially in the case of the young bugs hatched in the laboratory.

The mosquitoes were kept in glass jars as recommended by Christophers and Stephens in their *Practical Study of Malaria*. These jars were kept either in the laboratory or in the Sirdarb where the temperature was lower. In order to have a still lower temperature, mosquitoes were also kept in porous earthenware pots about six inches high and about five inches across. These were covered with mosquito netting and placed in a plate of water. The water from the plate soaked up the sides and by evaporation produced a temperature of about 70° to 75° F. These pots are of the same material as that from which the large earthenware filters or hubs are made, and it is on the moist cool surface of these hubs that the mosquitoes about the house repair during the hot part of the day. The small earthenware pots covered with mosquito netting reproduced the natural conditions very exactly. In

the pots the mosquitoes would sit on the sides, and of all methods for keeping them alive this was the best. There is constant moisture, a relatively low temperature, and a good supply of air. Of course, one has to be careful to keep the plate supplied with water, and further to keep all such jars out of the reach of ants which very soon destroy the flies and mosquitoes if they can attack them. House-flies and *Stomoxys* were kept in the same manner and also in wooden boxes with mosquito netting fronts.

For actual feeding on the sore the mosquitoes or flies were liberated in a mosquito net and taken up separately in small glass tubes ($3 \times \frac{3}{4}$ inch). These were inverted open mouthed over the sore, and, after feeding, the mosquitoes or flies were returned to the respective jars. In the glass jars the mosquitoes survived much longer if a clean sterilised jar and cork were used each day.

For breeding mosquitoes in the laboratory large mosquito nets were used and in these earthenware basins of water with the larvae were kept. Larvae were collected from the wells in small nets of mosquito netting fixed to the end of long bamboos.

E. *Character of the parasite as found in the sore.*

The parasite in smears from the sore may vary very much from the classical oval body with deeply staining rod and paler nucleus. The typical parasites are found in large numbers both within the large mononuclear cells which are probably of endothelial origin and also free in the plasma of the sore. In addition to these forms others occur in which there is considerable departure from the typical oval form. Dr Row has called attention to this fact and has figured many of these forms which are often much elongated and pointed at one end. In these elongated forms the kinetonucleus is often closely applied to the nucleus so that sometimes there is the appearance of the two having fused into one nucleus. In carefully stained films it is nearly always possible to distinguish the kinetonucleus even though it may be lying over the nucleus, and the number of forms where the two are indistinguishable can easily be explained by the fact that the kinetonucleus is under the nucleus and so rendered indistinct or invisible. A reference to Pl. XII, figs. 22–24 will show these conditions. I do not think the kinetonucleus and nucleus have fused in any of these elongated forms as Dr Row thinks must be the case. Nor do I think

that they represent a higher developmental condition than the ordinary forms. I rather think that these are forms endowed with greater powers of resistance and to them may be due the fact noted by Row that the parasites of the sore are able to survive a longer time in the scrapings from the sores than do the parasites of Kala azar in the material obtained by splenic puncture.

In these pointed forms the kinetonucleus is almost invariably between the nucleus and the blunt extremity, so that the tapering of the body is not a step in the direction of flagellum formation as one is inclined to suppose. These elongated forms, so aptly described by Dr Row as torpedo or cigar-shaped parasites, often occur in great numbers in the juice from the sore, and every transition between the typical oval body and the elongated narrow forms can be found. Amongst these may be seen some that have completely lost their chromatin, and appear in the film as homogeneous pinkish bodies of varying shape. Others occur in which the nucleus is lost while the kinetonucleus remains (Pl. XII, figs. 27 and 28). Such forms are undoubtedly degenerate or involution forms. The elongated forms reproduce by division as do the more typical ones. It will be seen that the appearance in a smear from a sore differ in many ways from smears of internal organs in Kala azar. In this latter disease there is a much greater uniformity in the structure and form of the parasites, and one does not encounter the curious elongated and involution forms. The intracellular parasites, the typical oval bodies, in the two cases are indistinguishable, but the appearances in the sore smear differ very markedly in the deviation of the parasite from this type.

The typical parasite (Pl. XII, figs. 25 and 26) as is well known, is an oval or bean-shaped body with the deeply staining rod-shaped kinetonucleus and the more brightly staining nucleus usually applied to one side of the parasite. The rhizoplast, first described by Mesnil, Nicolle and Remlinger in the parasite of Oriental sore in the same year that Christophers noted it in the parasite of Kala azar, is present in most cases. In well stained films it may be detected in almost all the parasites outside the cells, whether they are of the typical oval shape or elongated (Pl. XII, figs. 22–26). It is not easily detected in the intracellular forms probably because of the obscuring effect of the protoplasm of the cell. It may thus be said to be a typical feature of the parasite. In the dried films stained with Giemsa's stain, its connection with the kinetonucleus can be made out. The kinetonucleus stains very deeply except on one side where there is a paler staining

body. This appears to be part of the kinetonucleus and not a separate structure like the blepharoplast of some trypanosomes. It is from the margin of this paler body that the rhizoplast takes origin, whence it runs to the surface of the body. The first indication of a preparation for division is the growing out from this pale body of a second rhizoplast, parallel to the first (Pl. XII, fig. 26). This new rhizoplast is at first thinner than the original one, but it gradually increases in thickness till it attains the same size. Such a connection of the flagellum to the kinetonucleus I have seen in some of the reproducing forms of *Trypanosoma lewisi*. Too much weight cannot be attached to the appearances presented in dried films, but a similar connection can sometimes be made out in films, prepared by more rational methods. The protoplasm of the parasite is usually vacuolated to a greater or less extent and finely granular. Some of the granules may stain red with Giemsa's stain. The appearance of the nucleus in these dried films is well known and calls for no remarks beyond this, that the drying process has completely destroyed the normal appearance.

In films fixed in Schaudinn's fixative and stained by the iron haematoxylin method, the normal appearance is better retained. The parasites however are so minute, that it is difficult to make out clearly such details as the relation of the rhizoplast to the kinetonucleus. This is more clearly shown in some of the larger cultural forms. However, in films from the sore fixed and stained thus, one sees clearly that the nucleus is a spherical body limited by what appears to be a delicate membrane. At the centre of the nucleus is the deeply staining karyosome which varies in size according to the extent of extraction of the stain by the iron alum solution. The rhizoplast is as a rule very difficult to detect in these preparations. The kinetonucleus stains as a black rod (Pl. XIII, figs. 9 and 15).

Examinations of the sores made to discover if any morphological change occurred in the parasite as the sore developed, showed that all the forms described above could be found in the youngest as well as in the oldest ones. In the older sores that are commencing to heal, the most noticeable feature is a diminution in the number of parasites which eventually become very difficult to find.

In the non-ulcerating sores, the parasites occur in the large mononuclear cells. The nuclei of these cells are frequently riddled with parasites and may be completely destroyed by them. Portions of these cells are often broken off in the process of film making, and one has the appearance of several parasites embedded in an enucleate

mass of protoplasm. In the suppurating and ulcerating sores one frequently finds that the parasites have been taken up by the polymorphonuclear leucocytes.

F. *Culture of the parasite.*

The parasite of Oriental sore was first cultivated by C. Nicolle and later by Row in India. I first successfully cultivated the organism on a medium made after the formula of the blood agar of Novy and MacNeal with the substitution of dog's for rabbit's blood. Subcultures were made on dog's blood agar and on rabbit's blood agar. The development was never so rapid with the former as with the latter. Eventually the cultures on dog's blood ceased to develop. Possibly this may have had something to do with the natural resistance of the Bagdad dog to this disease as the blood was taken from the ordinary dogs off the street. On rabbit's blood agar cultures were maintained for some months. The development of the parasite in the cultures follows closely that given by other observers, so that it is not necessary here to enter into a detailed description of what takes place.

Dr Row has described the first process in the culture of the parasite as a fusion of the kinetonucleus and nucleus. I have not been able to confirm this. Many appearances are seen resembling those seen in the elongated forms from the sore where the intimate association of the kinetonucleus and nucleus may have the misleading appearance of complete fusion. I do not think that either in the sore or in the culture does a fusion of the two nuclei take place.

Dr Row mentions certain differences as existing between the cultures of the sore parasites and those of Kala azar. Apparently he compares his cultures of the sore parasite made on human blood serum with the culture of the parasite of Kala azar in citrated blood. It is not safe to emphasise such distinctions unless the conditions of culture are identical in the two cases. The appearances of the flagellates in dog's blood agar, for instance, show marked differences in size, shape, and rate of multiplication from those on rabbit's blood agar. C. Nicolle says that cultures of the two parasites under identical conditions show no differences. Dr Row's cultures were made with human blood serum which is perhaps an ideal medium for these parasites, and to this is probably due the rapid development and large size of his cultural forms.

G. *Character of the developmental forms of the sore parasite in blood agar culture.*

The parasites in cultures may be studied in dried films stained by Giemsa's stain, or better in films fixed without drying and stained by Heidenhain's iron haematoxylin method. Some of the flagellate forms as seen in dried films are shown at Pl. XII, figs. 30–36. In some of these the karyosome within the nucleus is shown in stages of division, but they are most interesting from the point of view of the flagellum which shows a connection with the kinetonucleus similar to that which was seen described above for the parasites from scrapings of the sore. In these it appears as if the kinetonucleus is a structure enclosed by a delicate membrane, on one side of which lies the deeply staining chromatin mass. From the opposite side of the membrane springs the flagellum, and it is from this membrane that a new flagellum grows out when division is about to take place. Sometimes the spot at which the flagellum unites with the possible membrane appears slightly enlarged and has the appearance of a blepharoplast.

The structure of the cultural forms is very well shown in films stained with iron haematoxylin after fixation in Schaudinn's fluid (Pl. XIII). It will be seen that the resting nucleus like the nucleus of a trypanosome consists of a delicate membrane enclosing a clear space at the centre of which lies a deeply staining karyosome. The kinetonucleus is a rod-shaped body staining black. The exact connection of the flagellum with this is difficult to trace. In some it appears to unite directly with the kinetonuclens (Pl. XIII, figs. 1, 8, 10, 13). In others it cannot be traced so far, while again it may be united by a faintly staining structure (fig. 11) resembling to some extent the appearances given in the dried films. There does not seem to be a separate blepharoplast as in many trypanosomes. Such a cone-shaped structure has been described and figured (figs. 4 and 5) by Robertson and Minchin in the collar cells of *Clathrina coriacea*, and I have shown a somewhat similar cone-shaped prolongation of the nuclear membrane in the case of *Cercomonas* in which the flagellum arises from the summit of the cone.

The various division stages are very clearly shown in the wet fixed films, especially in some of the short stumpy forms. The first indication of a division is the further elongation of the already rod-shaped kinetonucleus and the formation of a second rhizoplast parallel to the first. The earliest stages of the formation of the new rhizoplast

are not easy to trace owing to the difficulty of distinguishing it till it is of some thickness. Its connection with the kinetonucleus is the same as that of the first rhizoplast. In some of the dried specimens the new rhizoplast is seen growing out from a point on the surface of the pale half of the kinetonucleus as pointed out above (Pl. XII, figs. 31 and 34). The further division of the kinetonucleus is very clearly seen in the specimens fixed without drying. The elongated kinetonucleus becomes constricted towards the middle, and the two halves separate more and more, though they remain connected by a fine filament even up to the stage when the protoplasm of the parasite is commencing to divide (Pl. XIII, figs. 7, 8, 10, 14). Meanwhile, the new rhizoplast has been gaining in thickness and increasing in length till it commences to grow out from the surface in the form of a new flagellum. This new flagellum is at first thinner than the already existing one. At first the free extremity is closely applied to the original flagellum (Pl. XIII, fig. 4). I believe this appearance is due to the fact that the flagellum is enclosed in a delicate protoplasmic sheath, a continuation of the superficial ectoplasmic layer of the body. The new flagellum grows outwards within the sheath of the first flagellum and it is only when the new flagellum is fairly long that this sheath divides longitudinally so that each flagellum has its own sheath and can exercise independent movements. The new flagellum increases in length and thickness till when the division of the flagellate is complete it may not be equal in length to the original flagellum. I think that in the majority of trypanosomes the formation of a new flagellum takes place in a similar manner and the distal end of the new flagellum is within the sheath of the original flagellum, and often closely held to it so that the end may appear to unite. Such an appearance often gives one the impression that the new flagellum is forming by longitudinal division of the old one, but a careful examination will nearly always show that a slight interval exists between the termination of the new flagellum and the side of the old one. As the flagellum grows longer the part already formed may stretch the common sheath and bring about its division, so that there are two undulating membranes in the earlier formed portion, while the distal extremity is still within the common sheath, and closely applied to the old flagellum.

Eventually the filament connecting the two halves of the kinetonucleus ruptures and the division is complete. The length and fineness of this connecting filament are remarkable. It retains the stain intensely showing up even after much extraction by the iron alum solution.

One is inclined to think that it is an indication of some intranuclear division centre though this has not actually been observed. In these flagellates the flagellum is generally traceable back to the kinetonucleus itself, and does not appear to rise from an extra nuclear blepharoplast as in many trypanosomes. It is possible that such a blepharoplast may be in some cases within the kinetonucleus and may be represented here by the pale staining portion of the kinetonucleus from which the flagellum is seen to arise. In division it would be obscured by the surrounding chromatin of the kinetonucleus though the filament connecting the two halves is visible when the chromatin has divided and retracted from around the filament. In the division of the collar cells of *Clathrina coriacea*, so clearly described by Minchin and Robertson, the blepharoplast acting as an extra nuclear division centre takes on appearances very similar to these. The position of the blepharoplast outside the kinetonucleus as in trypanosomes of the blood, may be looked upon as a higher type of development while more primitive flagellates such as those under discussion, representing as they do flagellates of the intestinal tract of insects, display a more primitive condition with the blepharoplast within the nucleus.

To return to the division of the cultural forms of the sore parasites, we find that very soon after the kinetonucleus shows signs of approaching division the karyosome of the nucleus becomes elongated and with it the nuclear membrane. The length of the karyosome increases, a constriction appears and the karyosome may be divided into two parts (Pl. XIII, fig. 6) at a comparatively early stage. More frequently however the two halves of the karyosome remain connected by a filament which may eventually stretch across the whole width of the parasite. At this stage the nuclear membrane is seen surrounding the ends of the elongated structure (Pl. XIII, figs. 8, 10, 12, 16). The appearance of the karyosome thus elongated resembles very much, though on a larger scale, the condition of the dividing kinctonucleus. The size of the chromatin mass at each end of the structure varies with the extent of the extraction of the stain. The connecting filament however remains even after prolonged extraction (Pl. XIII, fig. 2).

It would appear that in the case of the nucleus also there is within the karyosome a division centre obscured by the chromatin, and in division represented by the fine connecting filament, this filament being both in the case of the kinetonucleus and nucleus a centrodesmose corresponding to the centrodesmose described by Minchin and Robertson in the case of the division of the collar cells of *Clathrina coriacea*.

Eventually the nuclear division is complete and two daughter nuclei formed. The division of the body of the flagellate has been proceeding by a groove appearing between the rhizoplasts. The presence of the filament connecting the two halves of the kinetonucleus appears to arrest the division of the protoplasm for some time, but when this filament is ruptured the division extends to the non-flagellate end of the body and the two daughter flagellates separate.

A point of much interest in connection with the culture of these organisms is the presence of extremely minute forms. One frequently encounters examples not more than 3 μ or 4 μ in length and about 1 μ to 2 μ in breadth, while on several occasions I have seen smaller individuals barely 2 μ long and not more than 0·5 μ in thickness. It is just possible in these small specimens stained with iron haematoxylin to make out the nucleus and kinetonucleus. The flagellum is relatively large. The presence of these minute forms and the possibility of others still smaller must be borne in mind when examinations of flies is made for developmental stages. It is possible that the form transmitted to man by the insect carrier is some such minute flagellate as this. They would be extremely difficult to detect amongst the debris frequently present in an insect's gut.

H. *Character of the development forms of the sore parasite in bed bugs and Stegomyia fasciata.*

The various developmental forms in the bed bug are shown in Pl. XII, figs. 1–21. Figs. 1, 5, 8, 10–21 are taken from a bug dissected forty-eight hours after feeding on the sore. The bug was opened and a dry film made from the contents of the stomach and another from the hind gut. The stomach showed a fair infection with flagellates. In the hind gut only a single flagellate was found. It will be seen that many of the forms correspond very clearly with those met with in the artificial cultures. Others however show various abnormalities and many appear to be degenerating. Figs. 10 and 14 show two such degenerating flagellates, while figs. 3, 9, 15, are examples of abnormal forms. Apparently the development is only an abortive one and a partial picture of what would take place in the true host. Figs. 2 and 6 are from another bug and figs. 3, 4, 7 and 9 from a third. Some of these appear to be normally constituted, while others, especially the curious form at fig. 9, are evidently abnormal. Figs. 38, 39, 41 show three parasites from a *Stegomyia* which had four feeds from the sore

and was dissected twenty-four hours after the last, while fig. 37 is from one that was fed on ten successive days and dissected forty-eight hours after the last feed, and fig. 40 from one that had two feeds and was dissected twenty-four hours after. There is nothing of special interest to note about these forms except their resemblance to the cultural forms met with in the test tube cultures.

I. *Attempts to transmit the sore by the bites of Stegomyia fasciata.*

Owing to the fact that the parasite of the sore develops in the *Stegomyia fasciata* and that this mosquito is so constantly attacking man, it was regarded as a very probable transmitter of the disease. Accordingly a series of experiments were undertaken in order to test this point. Specimens of *Stegomyia fasciata* were allowed to feed on the sore for a varying number of days and in each case after a lapse of twenty-four or forty-eight hours after the last feed from the sore the mosquitoes were allowed to feed on a small area about the size of a shilling on my forearm. In this way were fed twenty-six mosquitoes. Six of these had fed from the sore on ten successive days, and forty-eight hours later on my arm, fourteen had fed from the sore on four successive days and on my arm after an interval of twenty-four hours, two had fed from the sore on five successive days, and after twenty-four hours on myself; three had six successive feeds from the sore and a feed on my arm after an interval of forty-eight hours; one had fed once from the sore and after forty-eight hours on myself.

The following table gives the details of these feeding experiments:

Number of *Stegomyia*	Number of days on which *Stegomyia* had fed on the sore	Interval elapsed between last feed from sore and feed on myself
6	10	2 days
14	4	1 day
2	5	1 day
3	6	2 days
1	1	2 days

All these mosquitoes were examined twenty-four hours after they had fed from my arm. In two of those that had fed from the sore four times, and in one that had fed five times, were flagellates found. The rest gave negative results. Nearly nine months have elapsed since this experiment was made, and there is still no sign of a sore developing at the spot where these mosquitoes fed. All of them fed from the same small area of skin. I was exposed to infection all the time I was in

Bagdad, so that unless a sore had developed at the exact spot the experiment would be valueless and even had a sore developed there, there would be the possibility of some other fly having bitten and produced the sore. Still the failure of the *Stegomyia* to produce an infection seems to give some evidence against its being the natural host of the sore parasite. It would have been better had more than twelve days elapsed between the first feed from the sore and the feeding on myself, but it is only possible to form a single experiment on oneself, and the difficulty of finding persons suitable or willing for such experiments, prevented them being extended. The experiment is interesting in so far that it has given a negative result.

J. *Origin of the Disease.*

There are many local hypotheses regarding the origin of the sore. Some people maintain that it is only those who drink the water of the Tigris or Euphrates who become infected; others that it is due to the contamination of open sores or wounds by dirt from the roads, while others think it is in some way related to syphilis. The names "date boil" and "date mark" are attributable to the view that the disease is due to the dates which become ripe at the season of maximum incidence of the sore. None of these hypotheses will explain the disease. The peculiar distribution of the sore on the exposed surfaces of the body, the character of the specific parasite and the development of this parasite into *Herpetomonas* forms in the culture tube can only be accounted for by the assumption that there is some fly responsible for the transmission of the sore. The possibility of the house-fly acting as a mechanical carrier of the disease has been mentioned above. It is almost certain that the house-fly from time to time plays the part of such a mechanical carrier and transfers some of the parasites from an open sore to a fresh and healthy wound. The parasites may be carried over in moist material adhering to the proboscis or feet of the fly. That this mechanical transmission by the house-fly is to be regarded as the normal means of infection cannot be maintained, for it is certain that sores appear on parts of the skin where there have been no wounds or abrasions. It is improbable that the mere application of juice from a sore to the healthy skin will give rise to an infection, though it would be of interest to test this point experimentally. Even if it were possible for the mobile cultural forms of the sore parasite to pass through the uninjured skin, it is difficult to imagine how the passive immobile forms

found in the sores could do so. It is possible, as Dr Row suggests, that the parasite undergoes some development in the house-fly, and is then deposited upon the skin probably in the faecal matter, and that a sore arises in this way. Dr Row is conducting experiments with the object of elucidating this matter, but the quick disappearance of the parasites in the house-fly and their failure to develop into *Herpetomonas* forms, afford a strong argument against the view advanced by Row. The fact that the parasite develops into flagellate forms in the culture tube and also in the bed bug and in *Stegomyia* is almost conclusive evidence that a similar development will take place in the true host. From this point of view the house-fly cannot be suspected. However, in the light of the experiments of Hindle on the passage of *Trypanosoma gambiense* through the normal uninjured skin of the rat, it is a possibility to be considered that the parasite of the sore is taken up by the house-fly, that it develops into flagellates in its gut, probably minute forms like those described as occurring in the culture tube and not easily to be detected, and that in this condition it is passed on to the skin and is able to penetrate and produce a sore. My experiments lend no support to such a view, and should the house-fly be the normal carrier it still has to be explained why the disease is limited in its distribution, though the house-fly occurs everywhere.

Of the biting flies in Bagdad the *Stomoxys* as the carrier of the disease, is not to be suspected, as it is not only limited in its distribution, but also fails to give any development of the parasite in its gut, even after as many as ten feeds from the sore. The *Hippoboscidae*, the ticks, fleas and body lice, will not explain the disease. The two former on account of their seldom attacking man, and the two latter because they would give rise to sores on the unexposed rather than the exposed surfaces of the body. The bed bug on account of the development of the Kala azar parasite which takes place in its gut, and its supposed responsibility for the spread of this disease, was at first looked upon with suspicion. It was soon found that its distribution in Bagdad was not wide enough for this hypothesis to be correct, and further it would not explain the occurrence of sores only upon the exposed surfaces of the body. In spite of the impossibility of the bed bug being the host of the sore parasite, it has been pointed out above that a certain development of the parasite takes place in its gut. This observation is of great importance, for it shows that the development of the Kala azar parasite, which was found by Patton to take place in the same host, may be of a similar nature. The evidence afforded by the occurrence of such a

development is not sufficient to prove the bed bug to be the intermediate host. There is just the possibility in my experiments as well as in Patton's that the flagellates seen in the bed bugs are parasites peculiar to these insects, but I consider this highly improbable since the dissection of numbers of bugs which have not fed from a sore has failed to reveal these flagellate forms. It seems therefore that the large quantity of blood taken up by these insects when they bite is able to act as a culture medium for the sore parasites, without this culture indicating in any way that the development is the true development in an intermediate host.

The only other biting flies of any note in Bagdad are the mosquitoes and the so-called sand flies (*Phlebotamus*). Of the mosquitoes the *Stegomyia fasciata* both on account of its voracity and numbers would be looked upon with greater suspicion than the various species of *Culex*. This suspicion is increased when we consider the development undergone by the sore parasite in its gut, a development which does not take place in the other mosquitoes. At one time I was inclined to regard the *Stegomyia fasciata* as the probable agent of transmission. It is essentially a house mosquito and is most persistent in its attempts to obtain a feed of human blood. When it has completed its fill it quickly digests this apparently enormous quantity and is ready on the next day for another feed. It bites as readily by day as by night, so that one is never free from its attacks. Allowing about two months as the incubation period of the disease the time of maximum incidence of the *Stegomyia* corresponds with the time of maximum incidence of the sore. The result of the experiment upon myself with *Stegomyia fasciata* detailed above, appears to me to negative such a view, so that at present it is impossible to say whether the *Stegomyia fasciata* can or cannot act as the transmitting host of the sore parasite. It is still possible that some other mosquito less numerous and not so voracious as the *Stegomyia fasciata* will eventually be incriminated, but a more probable fly has yet to be excluded, in the sand fly (*Phlebotamus*). Unfortunately, experimental work was not undertaken with these flies till later in the year. This was just commenced when the unfortunate accident to my assistant and the destruction of part of the laboratory completely put a stop to the work for some time. When this was again resumed, it was not found possible to secure *Phlebotamus* in sufficient numbers for experimental work, so that I am unable to give any experimental results in support of this fly being the agent by transmission. Its numbers and distribution both as regards place and season

are compatible with this view. It is a fly that has been suspected in other places, so that until further work is undertaken the question must remain undecided. I hope during the coming summer to resume the experiments and to obtain some further light on this interesting but difficult question.

Conclusions.

1. Oriental sore as it occurs in Bagdad does not differ essentially from that of other places. There may however be a variation in the virulence and duration of the Oriental sore in different parts of the world.

2. The Oriental sore attacks practically all natives of Bagdad generally between the ages of 1 and 3 years. Newcomers usually become infected within a year or two after arrival.

3. Occasionally individuals may escape infection though exposed to it for years.

4. There may be one, two or three sores at one time. More rarely there are more. Sometimes there are as many as twenty-three and even greater numbers are talked of in Bagdad.

5. Whether single or multiple the Oriental sore rarely appears on any but an exposed surface of the body, *e.g.* face, fore-arm, leg, hand or foot.

6. One attack confers absolute immunity for the rest of life. It is possible that the sore of Aleppo may not produce absolute immunity against the sore of Bagdad. The same may be true of other places.

7. From inoculations from man to man it is demonstrated that the incubation period is about two months.

8. Inoculation with juice from a sore as in vaccination against smallpox produces a single sore at the point of inoculation only. From this it is concluded that in cases of multiple sore each sore is a separate inoculation by the transmitting agent or a subinoculation by a house-fly or by the individual himself.

9. There is a seasonal prevalence of the disease. Though they may appear at any time of the year, sores most usually make their appearance during the months of September, October and November at the close of the hot season.

10. The duration of the disease is from twelve to eighteen months.

11. The sore commences as a small papule which increases in size. It may then ulcerate and extend (female sore of Bagdad) or it may remain dry with a scaly scab on the surface (male sore of Bagdad).

There is little pain and no demonstrable constitutional disturbance associated with the disease.

12. In all true sores the typical parasite (*Leishmania tropica*) can be found unless the sore is in the final healing stage.

13. I have not been able to demonstrate the presence of the parasite in the peripheral blood either by direct examination of the blood or by the cultural method.

14. There is a much greater variety in the shape and size of the parasites in smears from the sore than in smears from the internal organs of cases of Kala azar.

15. The parasites obtained from young sores are of the same shape, size and characters as those obtained from old healing ones.

16. I have failed to demonstrate the presence of the sore in any domestic animal nor has the examination of one hundred and ten dogs revealed the presence of canine leishmaniosis in Bagdad. Kala azar in man is not known in Bagdad.

17. House-flies collected from the faces of children suffering from the open type of sore nearly always show the sore parasites in the gut. The parasites quickly degenerate and do not develop into flagellate forms.

18. House-flies must often act as mechanical carriers of the disease to open wounds.

19. Mosquitoes, *Stomoxys*, and bed bugs fed upon the sore are found to take up parasites.

20. Only in *Stegomyia fasciata* and the bed bug do the parasites develop into *Herpetomonas* forms. This is however no evidence that these insects are the natural carriers of the disease.

The same remark applies to the development in bed bugs of the parasites of Kala azar described by Patton. Development probably takes place because of the large quantity of blood taken up acting as a culture medium.

21. The transmitting insect is probably sometimes the house-fly and more usually either one of the mosquitoes or the sand fly (*Phlebotamus*).

22. The parasites of the sore develop into *Herpetomonas* forms in rabbit's or dog's blood agar as previously demonstrated by Nicolle and Row.

23. The inoculation of dogs and other animals (not monkeys) with juice from a sore or artificial culture on blood agar has failed in my hands to infect these animals.

24. No treatment has had much effect in reducing the duration of the sore.

25. Much can be done by protective inoculation on unexposed surfaces of the body not only to avoid the disfiguring scar on the face, but to prevent this having access to sores and thus becoming infective to other people.

II. The Life History of *Lankesteria culicis* (Ross, 1898).

Gregarine in *Stegomyia fasciata*.

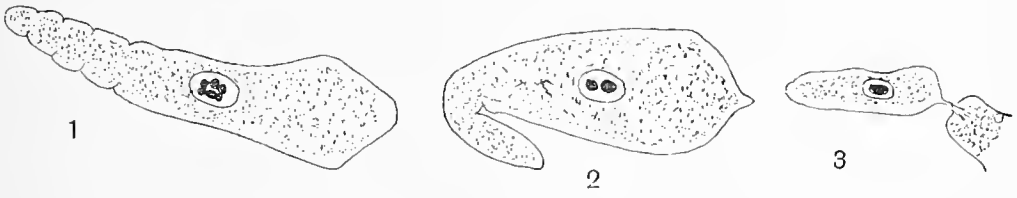
General account of the infection. Gregarines in this mosquito were first noticed and described by Ross during his classical researches into the development of malarial organisms on mosquitoes. The main features of the life history of this gregarine were described by him as follows.

"The youngest gregarines are found in the perivisceral cells of the youngest larvae. Growing in size, they escape from the host cell, become active and when the larva develops into a pupa, migrate into the malpighian tubes. There they become encysted with or without conjugation, and produce a large number of pseudonavicellae which are expelled with the faeces of the imago, either into water or upon the human skin." This gregarine was subsequently rediscovered by Marchoux, Salimbeni and Simond when conducting investigations on yellow fever. It is evidently the same gregarine which is to be found in many of the *Stegomyia fasciata* of Bagdad. Here the *Stegomyia* breed chiefly in the wells, and I have noticed that not all the wells are infected with gregarines to the same extent. In some wells practically every larva or pupa is infected, while in others only a small percentage and in some the *Stegomyia* appear to be free.

By a process of section cutting, somewhat laborious, of the body contents of the *Stegomyia* in all stages of its development I have been able to follow out almost the complete cycle of development of the gregarine. The body contents of larvae were dissected and placed one upon the other upon a slide kept moist under a glass cover, so as to form a small heap. When twenty to thirty had so been gathered into a heap the whole mass was fixed in Schaudinn's fixative. There was little tendency for the individual parts to separate so that it was possible to treat the collected organs as a single piece of tissue. This was washed, cleared and embedded in paraffin in the usual manner. The mass was then cut into serial sections which were stained with

haematoxylin. In this manner some beautiful preparations of the stages of development of the gregarine were obtained, and it was in this material that I have followed out the development. Unfortunately there are one or two gaps in this development owing to the fact that insuffieient material had been eollected, but eertain stages ean only be obtained by the examination of large quantities of material in the manner described above, a process whieh is very tedious.

In the gut the free forms are usually about 50 μ in length or smaller. I have not eneountered sueh large forms as those described by Ross as being 200 μ long. When examined in the living eondition



Lankesteria culicis of *Stegomyia fasciata*.

Figs. 1 and 2. Gregarines free in the gut of the larva of *Stegomyia fasciata*.
Fig. 3. Gregarine attached to epithelial cell.

they exhibit the typieal gregariniform movements of progression, flexion, and eonstriction. Sueh free forms are figured at Text-figs. 1 and 2. In sections of the gut it is found that the gregarines remain as intraeellular parasites during nearly the whole of their trophic development. Pl. XV, fig. 5 shows a gregarine eompletely within its cell, and fig. 8 another 50 μ long, also within the epithelial cell which shows a slight rupture at the surfaee. The general shape and features of the gregarine eall for no special remark. They are elearly shown in the figures. The body is non-separate and the single and typieal gregarine nueleus is at the eentre of the body. At the anterior end of the body is a peeuliar strueture which reminds one of the similar body described by Siedleeki in *Lankesteria ascidiae*. It appears to be a vesicle and is possibly a suetion organ eonneeted with the endoplasm through a pore in the euticle. It evidently functions as an organ of fixation whieh is used chiefly when the epithelial cell is eompletely ruptured and the gregarine remains attached to its degenerate remnants. The most eareful examination of the point of fixation of the gregarines in sections has failed to reveal any proeesses or fibres passing from the fixation organ into the epithelial cell. The gregarine lies in a vacuole in the epithelial cell and the fixation organ is merely

applied to that surface of the vacuole which is nearest the basement membrane. I have used the word suction, but it must be admitted that there is no indication of a stress being produced in the protoplasm which one would expect to find if a suction force were being exerted. In the case of tape worms fixed to the gut it is easy in sections to see that such a suction is taking place, for the tissues in the neighbourhood are dragged, as it were, towards the sucker. If we can compare a gregarine with such a large organism as a tape worm we might expect to find some signs of stress though on a smaller scale. Nothing of the kind can be detected, so it is possible that the peculiar organ which has been called a sucking organ, merely secretes some adhesive substance which will hold the gregarine in its place. As in *Lankesteria ascidiae*, immediately below this fixation organ is a deeply staining area from which pass fan-wise the fibres which run through the anterior part of the body of the gregarine (Pl. XV, figs. 5 and 8). These fibres are evidently contractile and possibly enable the gregarine to bring into action the fixation organ at its anterior end.

When the gregarine is about 50 μ in length, the epithelial cell ruptures and the gregarines remain attached to the epithelial remnants for some time. They then become detached and fall into the gut cavity where they exist for a while as free gregarines. After the passage of the *Stegomyia* larva into the pupa, as pointed out by Ross, the gregarines migrate into the malpighian tubes. The stimulus acting upon the gregarines is probably the sudden cessation in the intake of food by the *Stegomyia*.

Encystment. Within the malpighian tubes the gregarines associate in pairs, becoming attached to one another by the anterior ends, the two sucking organs coming into apposition. The point of contact of the two gregarines appears in the stained sections as a deeply staining area (Pl. XV, figs. 3 and 7). A similar appearance is shown by Siedlecki in the case of *Lankesteria ascidiae*. The two gregarines become enveloped by a thin membrane forming spherical cysts. The cells of the malpighian tubes become excavated to accommodate these comparatively large gregarine cysts. Pl. XV, fig. 9 is a longitudinal section of a malpighian tube of a *Stegomyia* pupa, showing the gregarine cysts and the alteration produced in the cells. Though in a single section it may appear that a single gregarine occupies one cyst, by following the sections through the series it is seen that there are always two. I have not encountered a case of a gregarine encysted singly.

Nuclear multiplication. The newly encysted gregarines usually have nuclei with a single large centrally placed karyosome. The karyosome soon becomes vacuolated and may break up into several fragments. At the same time the nuclear membrane becomes less distinct and the karyosome eventually passes into the protoplasm. In two cases, in close proximity to the nucleus, another structure has been seen which stains more deeply than the surrounding protoplasm. In Pl. XV, figs. 31 and 32 are shown in sections of a gregarine cyst the nucleus with the karyosome in process of breaking up in one section and in the next section the elongated staining body which shows some sign of radiation at one pole. Figs. 4 and 6 are two adjacent sections of another gregarine cyst. In one is seen the nucleus with the karyosome in two parts, and in the other a definite spindle forming with radiations around the two poles. At each pole is an area of denser tissue showing a few small vacuoles. This denser tissue which takes up the stain more deeply than the surrounding protoplasm is probably of centrosomic nature. For lack of material it is impossible to follow up the stage any further, but there is evidently a preparation for the formation of the first nuclear spindle. These stages bear some resemblance to the first spindle formation and the "achromatic mass" described for *Metamera schubergi* by Duke.

Though from the material at my disposal I was able to discover some trace of the first nuclear division, I was quite unable to find a cyst showing the second division. Various stages of the third division in a gregarine cyst are shown at Pl. XV, figs. 28, 29, 33, 34. In one gregarine the division was slightly more advanced than in the other. Figs. 33 and 34 are two dividing nuclei of one gregarine. There is a centrosome at each pole of the nuclear membrane which is still intact. Around each centrosome is a definite astral system. I was not able to detect a centriole within the centrosome. Probably had I stained with the iron haematoxylin method, further details would have been revealed, but all the sections were stained with ordinary alum haematoxylin. The chromatin within the nuclei at this stage is arranged irregularly. The nuclei in this gregarine were still spherical, but in the gregarine associated with it, the spindles were slightly more advanced (figs. 28 and 29). The centrosomes have separated and the spindle has become elongated. Definite fibres can be seen running from one pole to the other and at the centre are several chromatin masses. I am unable to say if at this stage there is a definite number of chromosomes. The nuclear membrane, much attenuated, is still

present. In the former of these two gregarines the karyosome (fig. 30) with two vacuoles was lying free in the protoplasm. Though the nuclear multiplication had advanced as far as the third division, it was still intact. Attached to it at one side is a peculiar club-shaped body. A similar structure occurred in the nucleus of another gregarine (Pl. XV, figs. 1 and 2). In subsequent divisions the definite character of these spindles is lost or they are too minute to be detected readily. Pl. XV, fig. 19 shows a section of a gregarine cyst where some of the nuclei of one gregarine are already arranged on the surface preparatory to gamete formation, while others presumably in the other gregarine are in division. In this nuclear division all that can be made out is the spindle-shaped structure without any astral system round the poles, or a visible centrosome. In the minute spindles the chromatin appears at first as a single mass at the centre. It divides into two, each half supplying the chromatin of a daughter nucleus. It is possible that even at this stage the centrosome, astral system and spindle fibres exist, but that they are too small to be clearly made out. As the nuclear divisions proceed some of the nuclei cease to have any share in the multiplication process and they remain as undividing nuclei in the protoplasm (Pl. XV, fig. 19). They take no part in the formation of the gametes and ultimately degenerate with the residual protoplasm left over when the gametes are formed. During the process of nuclear multiplication the bodies of the gregarines, at first clearly distinguishable, became interlaced owing to their protoplasm becoming vacuolated and thrown into folds and ramifications. In such a cyst as that figured at Pl. XV, fig. 19, it is impossible to trace the bodies of the two gregarines. Presumably the nuclei which are still dividing belong to one gregarine, while the others which have placed themselves on the surfaces of the ramifications in preparation for the formation of gametes belong to the other.

Gamete formation and conjugation. When nuclear division is complete, the nuclei arrange themselves on the surface of the ramifications into which the bodies of the gregarines have been produced. Pointed elevations of this surface are formed and into each bud a single nucleus enters. The buds are then separated as small gametes 3·5–4 μ in diameter. The nucleus of each gamete is placed eccentrically and beyond the nucleus the protoplasm is produced into a pointed protuberance (Pl. XV, figs. 12, 14, 17). The protoplasm of the gametes shows a marked reticular structure whereas the pointed eminence beyond the nucleus is quite clear and hyaline. It is possibly a condensed portion of the thin limiting ectoplasmic layer.

Though the gametes are equal in size, this is not the case with their nuclei, which are of two kinds. There are gametes with large nuclei and others with smaller nuclei. This difference in size of nuclei evidently has to do with a differentiation into male and female gametes. A similar difference in size of nuclei has been described by Swarczewsky in *Lankesteria sp.* and Brasil has described an almost identical differentiation in the case of the gametes of *Urospora lagidis.* The difference in size of nuclei is best seen in the conjugating gametes, all stages of which can readily be followed in the sections (Pl. XV, figs. 11, 13, 15). Pl. XV, fig. 10, shows a gregarine cyst containing zygotes and several masses of residual protoplasm, in which are seen some of the incompletely divided nuclei.

Development of sporocysts. The zygotes become elongated (Pl. XV, fig. 20) and each becomes enclosed in a sporocyst which is flattened at the poles. Within the sporocyst the protoplasm divides into eight sporozoites after the nuclear divisions have taken place (Pl. XV, figs. 21 to 27). The nucleus of the zygote is a spherical body with the chromatin arranged irregularly over the surface of the membrane. The first division takes place regularly at right angles to the long axis of the sporocyst. The nucleus becomes elongated and the chromatin aggregated at the poles in the form of bands. After division the nuclei so formed migrate to the ends of the sporocyst, where they undergo a second division in a similar manner and in a line parallel to that of the first division. The final division takes place in the line of the long axis of the sporocyst and at right angles to the line of the first and second nuclear divisions. The eight nuclei then become arranged round the equator of the sporocyst.

As a rule the whole process of development from the encystment to the formation of the ripe sporocysts takes place during the pupal stage of the *Stegomyia fasciata.* In the newly hatched adult mosquito one finds the malpighian tubes containing gregarine cysts filled with ripe sporocysts. Very soon the cyst walls enclosing the sporocysts disappear and the sporocysts are found lying free in the cavity of the malpighian tubes. Pl. XV, fig. 35, is from a longitudinal section of such a malpighian tube. The sporocysts make their way into the gut and are expelled, as Ross has described, with the faeces. Evidently these sporocysts escape into the water where the mosquito lays its eggs, and are taken up by the newly hatched larvae. I have not observed the escape of the sporozoites from the sporocyst in the gut of the larvae nor the infection of the epithelial cells in the earliest

stages. There can be, however, no doubt that this is the mode of infection.

The whole course of this development bears a very close resemblance to the development of *Lankesteria ascidiae* given by Siedlecki. It differs in that there is incomplete isogamy, as distinguished from complete isogamy, where both the size of the gametes and their nuclei are equal, the unequal size of the conjugating nuclei marking a differentiation into male and female gametes. In both cases the gregarines are intracellular during the greater part of their trophic period and they both have the same peculiar fixation organ. The resemblance is so close that both forms should be included in the same genus. The name for this gregarine of *Stegomyia fasciata* will then be *Lankesteria culicis* (Ross) 1898. I did not encounter gregarines in any other mosquito though these were taken from the same well in which were breeding the *Stegomyia* showing the largest percentage of infected individuals.

III. Some Observations on the Development of the Haemogregarine of the Leucocytes of the Dog.

Occurrence of the infection. It has already been mentioned in the first section of this report that practically without exception, all the Bagdad street dogs are found to harbour this parasite. I was able to perform autopsies on one hundred and ten dogs of all ages and it was nearly always possible to find the developmental forms of this parasite in the spleen or bone marrow. Sometimes the infection was a small one and several preparations had to be searched in order to discover a single cyst.

In other cases the infection was very large, so that numbers of cysts occurred in each squash preparation of either spleen or bone marrow. The cysts can readily be detected with a low power objective in simple squash preparations of the fresh organs. It seems that when once a dog is infected with this haemogregarine it remains infected for the remainder of its life. It is probable that the duration of life of the dogs is not great owing to the fierce struggle for existence which these dogs have to endure in the Bagdad streets. The appearance of the haemogregarine as it occurs in the dried blood films stained by any Romanowsky stain calls for no remarks. It has already been fully described by several observers. In films fixed by methods more rational than those of drying (*e.g.* by Schaudinn's fixative) the haemogregarines

show a somewhat different nuclear picture. Instead of the irregular red staining mass which often is produced into strands, evidently the result of drying and partial flattening on the slide, the nucleus is made up of a group of deeply staining (Heidenhain's iron haematoxylin method) masses of chromatin. The masses appear to be bound together by a paler reticulum. The masses of chromatin are so closely packed together that it is often difficult to make out any further structure. If, however, the differentiation in the iron alum solution is carried further, it will be found that the deeply staining masses become discoloured and that some further details can be detected. The nucleus will then be seen to consist of a ring of minute granules at the centre of which is a larger granule or karyosome. This is evidently an optical section of a vesicular structure. It is difficult to say if any definite nuclear membrane is present or not, but the regular arrangement of the granules in a ring suggests a nuclear membrane. I am inclined to the view that a definite membrane is present. Within the membrane between it and the karyosome can be seen some very fine granules. The nucleus of this haemogregarine is then a vesicular nucleus limited by a nuclear membrane on the surface of which in deeply stained specimens large staining masses obscure all other structure, while in more discoloured specimens the nuclear membrane is seen to be covered with small granules. At the centre is a definite karyosome, surrounding which are still fewer granules arranged on the nuclear reticulum.

Reproduction in the spleen and bone marrow.

It has generally been supposed that this haemogregarine reproduces almost exclusively in the bone marrow, but in the case of the Bagdad dogs, at any rate, the reproducing forms are found as commonly in the spleen as in the bone marrow.

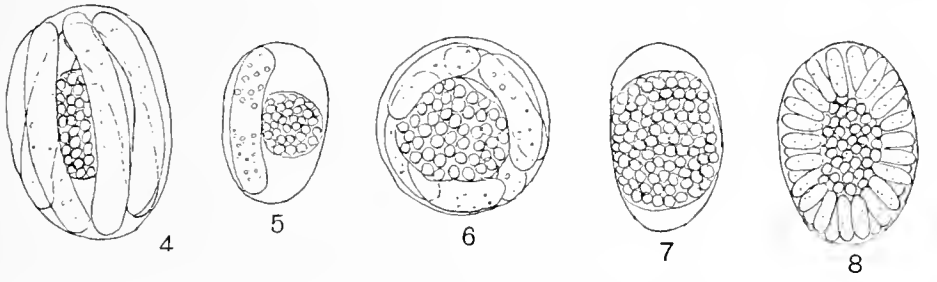
The reproduction follows two distinct lines. In one case, and this is the method which has hitherto been described, the resulting products of reproduction are numerous small bodies resembling the haemogregarines found in the leucocytes in the peripheral blood (Pl. XVI, fig. 8). In the other case, not described previously, the products of the reproduction are much less numerous (generally three) and very much larger, reaching a length of 15 μ (Pl. XVI, figs. 3 and 6). It may be stated at once that the small bodies are destined to enter the leucocytic cell in the peripheral blood, and that they are most probably gametocytes

which proceed with their development in the body of the tick *Rhipicephalus sanguineus*. The larger forms represent the asexual method of multiplication or schizogony.

A similar difference in the reproducing forms of other haemogregarines has been noted by Lutz, Prowazek, Reichenow and others, and I have described it in the case of *Haemogregarina gracilis* of the Sudan lizard, *Mabuia quinquitaeniata*. I suggested there that the small narrow forms which entered the red blood corpuscles and appeared in the peripheral blood were possibly sexual forms which would be found to pursue their further development in some intermediate host. This has been fully borne out by recent investigations. In their recent paper on the development of *Haemogregarina lutzi* Hartmann and Chagas describe a similar course of development. They mention the case of the *Haemogregarina gracilis* described by me, but they have stated that the so-called "microschizogony" products are the only form of asexual reproduction. In reality I said that the small forms are those destined to enter the red corpuscles and to appear in the peripheral blood while the large forms remain in the liver and reproduce asexually. The former are then probably sexual forms. The macroschizogony forms in the lizard as in the dog represent the asexual generation which occurs only in the internal organs, the so-called macromerozoites never appearing in the peripheral circulation.

The various stages of the development can be traced in sections of the spleen or bone marrow. In the present instance the tissue was fixed in Zenker's fixative and the sections stained with iron haematoxylin. The youngest forms are seen as small rounded bodies within mononuclear cells (Pl. XVI, figs. 4 and 13). The nucleus has a structure similar to that which has been described for the haemogregarines of the peripheral blood. The parasite increases in size and distends the host cell. At the same time the protoplasm alters in appearance, becoming filled with spheres of a refractile material. Very soon the nucleus commences to divide. The nuclear membrane is lost and the chromatin is arranged in an irregular mass of granules. With prolonged differentiation in iron alum solution it is seen that a karyosome is present in the midst of these granules. The karyosome divides after becoming elongated, and with its division the chromatin becomes collected into two masses each with its own karyosome (Pl. XVI, fig. 9). The karyosome acts in exactly the same manner as the karyosome of *Coccidium schubergi* in the dividing nuclei of the schizont as described by Schaudinn. Hartmann and Chagas have described a very similar nuclear division in the case of

the nuclear multiplication in the schizont of *Haemogregarina lutzi*. This process of division is repeated for each of the daughter nuclei or for only one of them. The nuclei resulting from the second division may again divide in a similar manner. There results a schizont filled with refractile spheres (Text-fig. 7) and having nuclei varying in number according to the exact progress of the nuclear divisions. From this are



Figs. 4–8. Reproductive cysts of *Haemogregarina canis* in the spleen of the dog, drawn from fresh material.

Figs. 4 and 6. Cyst containing merozoites and residual body drawn from fresh spleen material.

Fig. 5. Cyst containing one merozoite and residual body.

Fig. 7. Cyst containing schizont with protoplasm filled with refractile spheres.

Fig. 8. Cyst containing sexual forms with large residual body.

separated off large sausage-shaped merozoites having a fine reticular protoplasm while the refractile spheres are left behind in a large residual body. The number of merozoites correspond to the number of nuclei. In some cysts only a single merozoite is present (Text-fig. 5) with a large residual body. Here increase in size of the schizont can only have taken place without any nuclear multiplication, or only one of the nuclei resulting from the multiplication has given rise to a merozoite. Very commonly there are three merozoites but there may be four or twice this number (Text-figs. 4 and 6). The merozoites are about 15 μ in length. The nucleus varies in appearance with the extent of extraction of the stain. When fully extracted there is seen to be a nuclear membrane over which are arranged fine deeply staining granules. At the centre of the nucleus is a karyosome (Pl. XVI, figs. 3 and 6). I have not been able to decide whether the merozoites are able to again pass through a similar process of schizogony or whether each one passes on to the production of the sexual forms. I think the latter is more probably correct. If the asexual cycle could be repeated indefinitely, the infection of the dog would be much larger than it ever is. The dogs are covered with ticks and these ticks are constantly infected with the

sporozoites of the haemogregarine. Reinfection of the dogs must be constantly taking place, and sporozoites finding their way to the spleen or bone marrow. The probable course is that a sporozoite enters a mononuclear cell of the spleen or the bone marrow, increases in size, and eventually gives rise to a variable number of large merozoites which escape from their cyst and proceed to produce the small sexual forms which find their way into the peripheral blood. In favour of this view is the fact that it is very usual to find the cysts containing the small sexual forms grouped together in threes or fours in such a manner as to suggest that a sporozoite had recently produced three or four merozoites near this spot, that these have escaped from their cyst and settled down near together to produce the small sexual forms.

The process of formation of the small sexual forms takes place in a manner very similar to that of the merozoites with the difference that the nuclear divisions proceed very much further. The details are the same, and even up to the last division in suitably stained specimens the karyosome can be detected in the middle of the chromatin area (Pl. XVI, figs. 5 and 14). The number of nuclei is very large and there are produced a corresponding number of small sexual forms (Text-fig. 8). With the rupture of the cyst they escape and enter the mononuclear cells of the blood where they appear as the familiar haemogregarines in the leucocytes.

The host cell during both these processes of reproduction is reduced to a thin envelope surrounding the parasite. The nucleus of the host cell, very much flattened and altered, is seen at one side of the cyst (Pl. XVI, figs. 2, 8, 14). It is probable that in addition to this covering derived from the thinned out host cell the parasite secretes a covering of its own. These cysts are of some resistance, for in the squash preparations of the fresh organs they are not easily ruptured, and in the staining of smears which have been fixed without drying, the stain only penetrates with difficulty. The details of the contents of the cyst are only clearly made out in sections where the cysts have been opened at some point.

The fully formed cysts in the spleen and bone marrow are about 25–30 μ in diameter. The size of the cysts varies very little whether they contain the large merozoites or the numerous small sexual forms.

Further development of the leucocytic stage in the tick Rhipicephalus sanguineus.

The development of this haemogregarine in the tick was first described by Christophers. The method of investigating the development adopted by him, viz. the making of smears of the contents of the ticks, was not the best for giving clear pictures of what was taking place. By making smears it is impossible to avoid breaking up the large öocysts and scattering the sporocysts. In this way Christophers appears to have described the sporocysts as öocysts.

In the present instance the following method was adopted. Ticks were taken from infected dogs and the abdomens were opened by making a cut round the margin with fine scissors. By careful dissection with needles it was possible to remove the ventral chitinous plate intact, leaving all the organs behind. The organs were then removed in one piece and immediately fixed in Zenker's fixative. These fixed organs were brought home for examination. Serial sections were cut and these stained with haematoxylin. In these sections it was possible to follow many of the stages of development of the haemogregarine.

The first step in the development has been clearly described by Christophers and consists in the liberation of the cysts from the leucocyte and finally the escape of the haemogregarine from the cyst. The free haemogregarines can be found in numbers in the intestinal contents of the ticks.

I have also been able to trace haemogregarines within the epithelial cells of the gut and also between the epithelial cells and the basement membrane. Finally they are to be found outside the basement membrane amongst the body contents, usually, however, close to some fold of the gut.

Unfortunately, I have not yet been able to follow out the conjugation process.

For haemogregarines this has been most clearly described by Reichenow for *H. stepanowi.* The haemogregarines taken into the gut of the leech associate in pairs, and still within the gut a process of conjugation takes place which is very similar to that of the coccidian *Adelea ovata.* In one of the haemogregarines the nucleus divides into several parts, and one of these parts unites with the entire nucleus of the other haemogregarine. The remainder of the divide nuclei are discarded. After this the zygote increases in size and breaks up into sporozoites which pass through the gut wall of the leech into the

surrounding tissue. Robertson has also described a similar method of conjugation in the case of another haemogregarine *H. nicoriae.* The sexual process for the dog haemogregarine was described by Christophers, but Reichenow is of the opinion that here also the conjugation will ultimately be found to be of the *Adelea* type.

Miller, however, has described for the rat haemogregarine a complete conjugation of two unaltered haemogregarines in the gut of the mite *Lelaps echidninus.* It is possible that here the conjugation will be found also to be on the lines of that of *Adelea ovata.* Further work alone will settle the question of the exact details of the conjugation of these leucocytic haemogregarines of the dog and rat.

I have mentioned that I was able to trace the haemogregarines through the epithelium of the gut into the tissues of the body of the tick, but the forms I have seen might have been öokinetes passing through the gut wall after conjugation as do the öokinetes of the rat haemogregarine according to Miller, or they may have been even the sporozoites resulting from a sporozony undetected by me in the gut of the tick, as described by Reichenow and Robertson.

In order to follow in detail the stages which occur after the haemogregarines have reached the body tissues of the tick, one must have recourse to artificial infections. The ticks with which I conducted these investigations were taken from naturally infected dogs in which the infection was never large enough to enable me to follow in detail the next few stages. It is sometimes very difficult to distinguish the very young öocysts from the tissue cells of the ticks.

The next stage which I have been able to distinguish clearly is shown at Pl. XVI, fig. 11. Here it will be seen that a large cell has been produced, that nuclear multiplication has taken place, till there are present about thirty nuclei. I am not quite clear whether this is a growing öocyst or whether it is some stage in the production of gametes as described by Christophers. Miller has described for the rat haemogregarine an enormous increase in size of the öocyst. A similar increase in size takes place in the case of the dog haemogregarine. Eventually large öocysts of about 100 μ in greatest diameter are produced. These are found in the sections to lie outside the gut wall embedded in the surrounding tissues. These öocysts contain at first a single mass of protoplasm with thirty to fifty nuclei. By a process of budding (Pl. XVI, fig. 17) there are separated off protoplasmic masses each with a single nucleus. A great part of the protoplasm is unused in this process and is left over as a residual body. The protoplasmic

masses are really sporoblasts, and they soon secrete a covering of sporocyst. Within the sporocyst nuclear multiplication again takes place, till there are produced about sixteen nuclei in each (Pl. XVI, fig. 1). The nuclei then range themselves in groups, eight at each pole. By a process of growing out from the protoplasmic mass there are formed from each end eight sporozoites (Pl. XVI, fig. 7). A part of the protoplasm remains as a residual body. In this manner there are produced large öocysts of about 100 μ in greatest length containing from thirty to fifty sporocysts of about 15 μ–16 μ in length, and in each sporocyst sixteen sporozoites of about 14 μ in length (Pl. XVI, fig. 16). The sporozoites (Pl. XVI, fig. 12) have been described by Christophers. They are elongate bodies of 14 μ–15 μ in length and are packed tightly together within the sporocyst. The nuclei of the sporozoites resemble those of other stages of the haemogregarine. There is a mass of chromatin granules closely bound together. I have not been able to determine if a nuclear membrane or karyosome exists in this stage, but as the earliest forms found in the splenic cells have nuclei of this type, I do not doubt that the sporozoites have also.

The presence of these large öocysts was not noticed by Christophers, probably owing to his method of studying the development. In making smears of the contents of ticks these large öocysts would certainly be ruptured and the sporocysts liberated. In sections the general arrangement of the various parts is much better retained. The production of the öocysts and sporocysts corresponds in almost every detail with the development given by Miller for the very similar haemogregarine of the rat. The average number of sporozoites in each case is sixteen. The number of sporozoites in each sporocyst in the case of *Haemogregarina stepanowi* and *Haemogregarina nicoriae* is only eight.

The next stage in the development of the large öocysts in the dog tick is the dissolution of the cyst wall and the liberation of the sporocysts which wander about amongst the body contents of the ticks. Liberated sporocysts may be found in any part of the body outside the gut. In sections of ticks at this stage it is possible to find sporocysts in almost every section. The further development has not yet been traced. I have not been able to detect free sporozoites either in the body cavity or in the gut. In the case of the *Haemogregarina stepanowi* and in *Haemogregarina nicoriae* Reichenow and Robertson have described the liberation of the sporozoites from the sporocysts and their passage back into the cavity of the gut, whence undoubtedly they again enter the body of the tortoise. Christophers describes free sporozoites

in the gut contents, but in the method of smearing it is almost impossible to be sure from where the various forms seen have originally come. In the case of the rat haemogregarine Miller has not noted any escape of the sporozoites from the sporocysts while they are still within the body of the mite. He has found, however, that when treated with the intestinal juice of rats the sporocysts rupture and the sporozoites escape. This led him to feed healthy rats on crushed mites containing sporocysts. In this way, and in this way alone, was he able to produce an infection in healthy rats. It is possible that in the dog also infection takes place in a similar manner, through the intestinal wall, by the dog eating the infected ticks. The sporozoites would then be liberated in the gut, make their way into the spleen or bone marrow, enter a mononuclear cell and develop into the schizonts producing about three large merozoites. The merozoites would escape and develop into those forms which produce the well-known haemogregarines found in the peripheral blood. Whether these are really gametes or gametocytes cannot be settled till the exact process of conjugation is known. According to Miller they must be regarded as gametes which conjugate in the intestine of the tick.

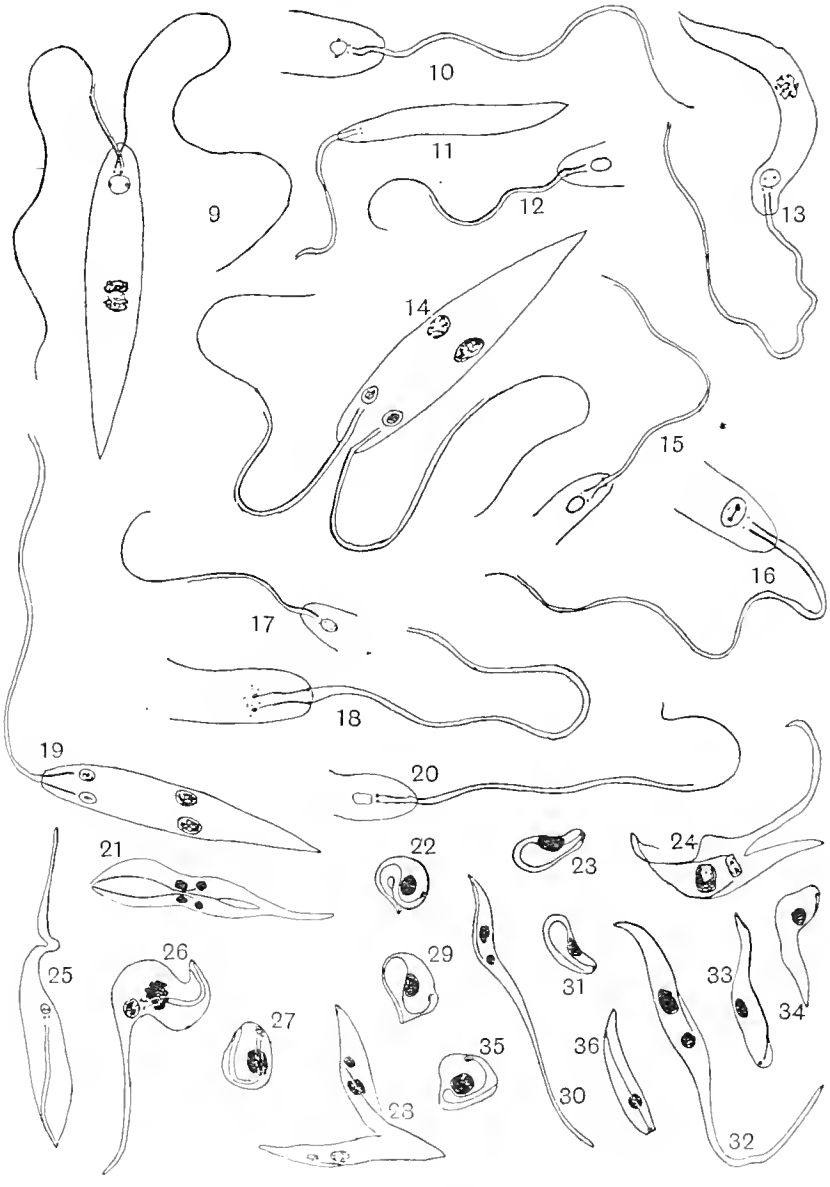
The course of the development given above for the haemogregarine of the dog, as far as it is complete, agrees in almost every detail with that of Miller, for the similar parasite of the rat. I hope at a later date to be able to fill in the gaps in this life history.

IV. Flagellates of House-flies.

As in many of the Eastern cities a large proportion of the Bagdad house-flies are found to harbour intestinal flagellates. Of these I have met with three types, but whether each type represents a distinct species of flagellate or three different stages of one and the same flagellate, I am not in a position to state. Two of these correspond with the flagellates described by Flu from the house-fly, and one is found chiefly in the malpighian tubes and is interesting in that it shows the trypanosome arrangement of nucleus and kinetonucleus. Of the two flagellates described by Flu one is the *Herpetomonas muscae domesticae* of Prowazek ; the other is spoken of by Flu as a *Leptomonas*. The chief object of the present account is to show that in the case of the former the description of Prowazek is probably based on a faulty interpretation of the appearance of the flagellate in division. Prowazek described his

flagellate as having a peculiar arrangement of the flagella. Each flagellate had a nucleus and kinetonucleus, the latter being often elongate with the long axis parallel to that of the body. Two rhizoplasts were present and each terminated at the anterior end of the body in a small granule termed a diplosome. From each diplosome arose a flagellum. The two flagella were attached to one another by some connecting substance or a membrane between them. In division one flagellum goes to each daughter flagellate and a new flagellum is formed by forward growth from the diplosome while the new rhizoplast grows backwards from the same structure. Patton has shown that in this flagellate the biflagellate arrangement is merely a stage in the longitudinal division. This is quite correct and I think that the various stages in this division which I have found completely establish Patton's contention. Mackinnon and Porter also have come to a like conclusion and agree with Patton that the flagellate of the house-fly is not a biflagellate, but that the appearances described by Prowazek are merely division stages.

A reference to the Text-figures 9 *et seq.* will make this matter clear. It will be seen that in all essential respects this flagellate (figs. 9 to 20) agrees with that of Prowazek and Flu. All the figures are drawn from films made from house-flies in which active multiplication of the flagellate was taking place. In other house-flies such a multiplication may not be in progress and the flagellate is then found with an elongated body, a nucleus and kinetonucleus which is much longer than broad, and a blepharoplast near the kinetonucleus from which arises the single rhizoplast which is continued into the single long flagellum. A vacuole is frequently present near the kinetonucleus and in many cases at the junction of the rhizoplast and flagellum is found a granule or enlargement of either of these structures which corresponds with the diplosome of Prowazek and Flu. The blepharoplast as a structure distinct from the kinetonucleus was not noted by Prowazek, and this is the only difference between my flagellate and such forms as figured by him. When house-flies in which active multiplication is in progress are examined, forms with a single flagellum may never be found and this is dependent upon a precocious flagellum formation, each flagellate being as it were in a hurry to have the new flagellum ready for a succeeding division. The result of this is that all the flagellates have either two or more flagella. A flagellate which has just resulted from longitudinal division has already two flagella arranged as shown in fig. 17. The kinetonucleus is an oval body limited apparently by a membrane. It



Text-figs. 9—36. *Flagellates of the House-fly.*

Figs. 9—12. *Herpetomonas muscae domesticae.*

Fig. 9. Dividing flagellate. The kinetonucleus shows the deeply staining granule on each side. There are two blepharoplasts and from one a new rhizoplast and flagellum are forming.

Fig. 10. Anterior end of another flagellate similar to fig. 9.

Fig. 11. Flagellate without nucleus or kinetonucleus. The two blepharoplasts and flagella are still present.

Fig. 12. Anterior end of a flagellate showing granules at the junction of the flagella and rhizoplasts. These are probably Prowazek's diplosomes.

Fig. 13. Dividing flagellate. Within the kinetonucleus are the two granules which have not yet reached the sides of the kinetonucleus which appears to have a definite membrane. The two blepharoplasts clearly shown.

Fig. 14. Dividing flagellate showing well the precocious formation of flagella. Though division is not complete the daughter flagella of the succeeding division are already forming.

Fig. 15. Anterior end of a dividing flagellate showing blepharoplasts, rhizoplasts and diplosomes.

Fig. 16. Anterior end of a dividing flagellate showing the dividing karyosome (?) within the kinetonucleus.

Figs. 17–20. Dividing flagellates. Fig. 18 from a flagellate in which the kinetonucleus has been destroyed. It shows well the union of the rhizoplast and blepharoplasts by means of a faintly staining line.

Figs. 21–36. *A flagellate of trypanosome type from the malpighian tubes of the house-fly. They possibly represent forms of* Herpetomonas muscae domesticae.

Fig. 21. Dividing form.

Figs. 22, 23, 27, 29, 31, 33–36. Various stages in the formation of the small pear-shaped forms (fig. 23) from the long forms (figs. 28 and 30).

Fig. 24. Large dividing form.

Fig. 25. Long dividing form without nucleus but still possessed of kinetonucleus and blepharoplasts.

Fig. 26. Dividing form showing two granules within the kinetonucleus and two blepharoplasts.

Fig. 28. Dividing form.

Figs. 30 and 32. Typical long forms. Note the long drawn out flagellar extremity of the body. The kinetonucleus is always on the non-flagellar side of the nucleus. There is no free flagellum as this structure terminates at the blunt end of the body.

is still single but close to its flagellar side are the products of the already divided blepharoplast. From the new blepharoplast a new rhizoplast narrower than the old one has formed, and this has grown out into the beginnings of a new flagellum. Patton believes that the new rhizoplast is divided off from the fresh one, but it is certainly a new formation growing out from the divided blepharoplast. The blepharoplast alone divides. Porter claims to have watched the division of the flagellum in the living *Herpetomonas muscae domesticae.* From the stained smears examined by me, I can find no evidence of such a division. The flagellum is enclosed, as I believe, in a thin sheath of ectoplasm continued from the body of the flagellate and it is within this sheath that the new flagellum grows out. As the flagellum increases in length both it and the rhizoplast increase in thickness till eventually the length and thickness equal those of the old flagellum. Such stages may be followed in figs. 17, 12, 10, 13, 16, etc. When the new flagellum has attained a certain degree of development, indications of division appear in the kinetonucleus. Within this there is apparently a structure like a karyosome.

This first divides into two equal parts (figs. 16 and 13). Each half then passes to one side of the kinetonucleus (figs. 9 and 10) and then the kinetonucleus itself is divided. Apparently the dark staining body which is within the kinetonucleus functions as a centrosome. The details of this division have been described but it must be mentioned that the films were dried ones stained by Giemsa stain. It is just possible that some of the appearances are the result of bad fixation, but they are so constant and occur in so many of the dividing flagellates that this can hardly be the case, though one has to exercise caution in drawing conclusions. After division of the kinetonucleus, division of the nucleus takes place, but it must be stated that the kinetonucleus does not divide till the new flagellum is equal or almost equal to the old one in length and thickness, so that many of the flagellates appear in the preparations with single nucleus and kinetonucleus, and two equal flagella which are bound together by some connecting material which is, according to my interpretation, the common flagellar sheath. These forms correspond exactly with Prowazek's figures and many of them show clearly the granule or diplosome at the base of each flagellum (figs. 18, 15, 10, 20). Just before or at the time of division of the kinetonucleus the common sheath which binds the two flagella together is divided longitudinally, so that the flagella may move independently of one another (fig. 9). Immediately after division of the kinetonucleus each blepharoplast divides again, a new rhizoplast is formed and a new flagellum grows out in such a way that these dividing flagellates have four flagella. This condition is very well shown in the flagellate drawn at fig. 14 and it corresponds very closely with some of Prowazek's figures of this stage.

Occasionally the formation of the new flagella may commence before the division of the kinetonucleus is completed, but this is a rare occurrence (fig. 9). The forms with four flagella are not numerous, for division of the two nuclei is quickly followed by division of the body of the flagellate. The result of this process of division is that none of the flagellates are uniflagellate, for at the time of division each half is provided with one fully formed flagellum and one partially formed one (fig. 17). It thus comes about that during the active multiplication uniflagellate forms are not to be found, and I think that this fact has undoubtedly led to Prowazek's interpretation of this flagellate as being a biflagellate. When, however, active multiplication is not in progress the flagellate is often found with a single flagellum. This is the resting form and every transition between the uniflagellate elongated forms

and the small oval forms with only a short flagellum or no flagellum whatever may be followed.

During multiplication, therefore, the biflagellate appearance is the result of a very active multiplication of flagella, a multiplication which appears to lead the way in longitudinal division of this flagellate. The protoplasmic body of the flagellate is reluctant to divide so that it is always behindhand and has not completed its division till after the daughter flagella of the succeeding division have partially developed. When the daughter flagella of the succeeding division have partially formed the flagellate, as it were, realises its backwardness, and tries to regain lost ground by very rapid division. This latter fact accounts for the comparative rarity of such forms as that shown at fig. 14.

The flagellate just described agrees with that of Prowazek in dimensions, and in most of the details of structure. The blepharoplast or achromatic structure near the kinetonucleus was not described by him, and I have failed to find the fibre which Prowazek traced through the body of the flagellate from its kinetonucleus. Flu has not figured this fibre. I think there can be no doubt that the flagellate here described is *Herpetomonas muscae domesticae,* and is the same as that described by Prowazek and Flu. According to my observations this flagellate agrees in all essential points with the other flagellates so often described as *Herpetomonas* and is not a biflagellate as maintained by Prowazek and others.

In addition to this large *Herpetomonas* a smaller one may be found in some flies. It corresponds with the flagellate described by Flu as a *Leptomonas.* In reality it should be included in the same genus as the larger flagellate of the fly. It may be a distinct flagellate as Flu claims, or it may be a stage in the life history of *Herpetomonas muscae domesticae.* In some flies the small form alone is found. In others there is a mixed infection with the large and small forms.

Still a third type of flagellate was found in some flies. These are shown in Text-figures 21–36. They occur mostly in the malpighian tubes, but may also be found in the gut. They are remarkable in showing a trypanosome arrangement of the nuclear apparatus.

There is no free flagellum, but this organ terminates at one extremity of the body, the opposite extremity of the body being drawn out into a long tapering filament (figs. 25, 26, 30, 32). The nucleus is situated in the thicker part of the body and the kinetonucleus between it and the tapering extremity. The flagellum arises from a blepharoplast lying near the kinetonucleus (figs. 25, 26, 28, 30), and is continued past the

nucleus to the blunter extremity of the body. There does not appear to be a true undulating membrane and the flagellum runs a fairly straight course over the surface of the flagellate. Dividing forms are shown at figs. 21, 24 and 26. In division the blepharoplast divides, a new flagellum is formed, after which nuclear divisions take place succeeded by division of the flagellate. Within the kinetonucleus there appears to be a karyosome which first divides as in the case of *Herpetomonas muscae domesticae* described above. A curious feature about this flagellate is the formation of small pear-shaped bodies (fig. 26) which may be stages in cyst formation. Actual cysts were not observed.

The first step in this process is the formation of small stumpy forms (figs. 33, 34, 36), some of which resemble *Trypanosoma nanum* very closely. The stumpy forms have the kinetonucleus at the blunt extremity of the body and they appear to arise by the gradual loss of the long tapering end of the long forms. The small forms then become doubled upon themselves (fig. 22), and finally the space between the two limbs is filled in and forms such as those shown at figs. 27, 29, 35 are produced, in which the flagellum follows a curious course through the body. Finally the body narrows and the small pear-shaped forms result (figs. 23, 31). It is possible that this flagellate also is some stage in the development of *Herpetomonas muscae domesticae*, but I have no facts on which to decide this point.

REFERENCES.

Basile, C. (1911). Sulla Leishmaniosi et sul suo modo di transmissione. *Rendiconti d. R. Accad. d. Lincei*, xix. pp. 523–527 ; xx. No. i. pp. 50–51 ; No. ii. pp. 50–51 ; No. iii. p. 7.

Brasil, L. (1905). Recherches sur la réproduction des Grégarines monocystidées. *Arch. Zool. expér.*, Ser. iv. iii. p. 17.

—— (1905). Nouvelles recherches etc., *Ibid.* iii. p. 69.

Christophers, S. R. (1906). *Leucocytozoon canis. Sci. Mem. Officers Med. and San. Dept. Govt. India.*, n. s. No. 26.

—— (1907). The sexual cycle of *Leucocytozoon canis* in the tick. *Ibid.* No. 28.

Christophers, S. R. and Stephens, J. W. W. (1908). The practical study of malaria. *The University Press of Liverpool ; Williams and Norgate, London.*

Donovan, C. (1906). Kala Azar in Madras. *Trans. Bombay Med. Congress.*

Duke, H. L. (1910). Some observations on a new gregarine (*Metamera schubergi*, nov. gen., nov. spec.). *Quart. Journ. Microsc. Sci.*, lv. pp. 261–286.

Dyé, L. (1905). Les Parasites des culicides. *Arch. de Parasitologie*, ix. pp. 5–77.

Flu, P. C. (1908). Ueber die Flagellaten im Darm von *Melophagus ovinus*. *Arch. f. Protistenk.*, xii. pp. 147–153.

FLU, P. C. (1911). Studien über im Darm der Stubenfliege, *Musca domestica*, vorkommenden protozoären Gebilde. *Centralb. f. Bakt, etc.*, LVII. pp. 521–534.

HARTMANN, M. and CHAGAS, C. (1910). Vorläufige Mitteilung über Untersuchungen an Schlangenhämogregarinen. *Arch. f. Protistenk.*, XX. pp. 351–363.

HINDLE, E. (1911). The passage of *Trypanosoma gambiense* through mucous membranes and the skin. *Parasitology*, IV. pp. 25–28.

MACKINNON, D. L. (1910). *Herpetomonas* from the alimentary tract of certain dung-flies. *Parasitology*, III. pp. 255–274.

MARCHOUX, SALIMBENI, and SIMOND (1903). La fièvre jaune. Rapport de la mission française. *Ann. de l'Inst. Pasteur*, XVII. p. 713.

MILLER, W. W. (1908). *Hepatozoon perniciosum* (N. G., N. Sp); a haemogregarine pathogenic for white rats: with a description of the sexual cycle in the intermediate host, a mite (*Lelaps echidninus*). *Treasury Dept. Public Health and Marine Hospital Service of the United States, Hygienic Laboratory Bulletin*, No. 46.

NEUMANN, R. C. *Leishmania tropica* im peripheren Blute bei der Dehlibeule. *Centralbl. f. Bakt. etc.*, I. Abt. Vol. LII.

NICOLLE, C. and MANCEAUX, L. (1910). Recherches sur le bouton d'Orient. *Ann. de l'Inst. Pasteur*, XXIV. pp. 673–720.

NICOLLE, C. and COMTE, C. (1908). Origine canine du Kala azar. *Compt. rend. Acad. Sc.*, CLXVI. p. 789.

NICOLLE, C. (1908). Culture du parasite du bouton d'Orient. *Ibid.* CXLVI. p. 842.

PATTON, W. S. (1907). The development of the Leishman-Donovan parasite in *Cimex rotundatus*. *Scient. mem. by officers of the med. and san. dept. of the Govt. of India*, No. XXVII.

—— (1908). The development of the Leishman-Donovan parasite in *Cimex rotundatus*. Second report. *Ibid.* No. XXX.

—— (1908). *Herpetomonas lygaei*. *Arch. f. Protistenk.*, XII. pp. 131–146.

PORTER, A. (1909). The life-cycle of *Herpetomonas jaculum*. Leger, parasitic in the alimentary tract of *Nepa cinerea*. *Parasitology*, II. pp. 367–391.

PROWAZEK, S. v. (1904). Die Entwicklung von *Herpetomonas*, einem mit den Trypanosomen verwandten Flagellaten (Vorläufige Mitteilung). *Arb. a. d. Kaiserl. Gesundh.*, XX. pp. 440–452.

REICHENOW, E. (1910). *Haemogregarina stepanowi*. Die Entwicklungsgeschichte einer Hämogregarine. *Arch. f. Protistenk.*, XX. pp. 252–350.

ROBERTSON, M. (1911). Life cycle of *Haemogregarina nicoriae*. *Report of the Advisory Committee for the Tropical Diseases Research Fund. Wyman and Sons, Fetter Lane, London*, pp. 33–34.

ROBERTSON, M. and MINCHIN, E. A. (1910). The division of the collar-cells of *Clathrina coriacea* (Montagu); a contribution to the Theory of Centrosome and Blepharoplast. *Quart. Journ. Micros. Sci.*, LV. pp. 611–640.

ROSS, R. (1906). Notes on the parasites of mosquitoes found in India between 1895 and 1899. *Journ. of Hygiene*, VI. pp. 101–111.

ROW, R. (1909). The development of the parasite of oriental sore in cultures. *Quart. Journ. Micros. Sci.*, LIII. p. 747.

—— (1909). Observations on the development of flagellated organisms from the parasite of "oriental sore." *Trans. Bombay Med. Congress*, pp. 204–208.

Row, R. (1910). Further observations on *Leishmania tropica* of Oriental sore of Cambay, India. *Brit. Med. Journ.*, No. 2595, p. 867.

Sambon, S. W. (1908). The haemogregarines of Snakes. *Journ. of Trop. Med. and Hyg.*, xi. and xii.

Schellack, C. (1907). Über die Entwicklung und Fortpflanzung von *Echinomera hispida* (A. Schn.). *Arch. f. Protistenk.*, ix. pp. 297–345.

Siedlecki, M. (1899). Über die geschlechtliche Vermehrung der *Monocystis ascidiae* R. Lank. *Anz. d. Akad. Wiss. Krakau.*

Swarczewsky, B. (1910). Beobachtungen über *Lankesteria sp.*, eine in Turbellarien des Baikalsees lebende Gregarine. *Festschrift zum sechzigsten Geburtstage Richard Hertwigs*, vol. i. pp. 637–674.

Wenyon, C. M. (1909). *Haemogregarina gracilis*. *Report of Travelling Pathologist. Third Report Wellcome Res. Lab.* p. 153.

—— (1910). Some observations on a flagellate of the genus *Cercomonas*. *Quart. Journ. Micros. Sci.*, lv. pp. 241–260.

—— (1911). Report of six months' work of the expedition to Bagdad on the subject of oriental sore. *Report of the Advisory Committee for the Tropical Diseases Research Fund. Wyman and Sons, Fetter Lane, London*, pp. 47–55 and *Journ. of Trop. Med. and Hyg.*, xiv. pp. 103–109.

Werner, H. (1908). Über eine eingeisselige Flagellatenform im Darm der Stubenfliege. *Arch. f. Protistenk.*, xiii. pp. 19–22.

EXPLANATION OF PLATES XII TO XVI.

PLATE XII.

Figs. 1–21. *Developmental forms of the sore parasite* (Leishmania tropica) *in bed bugs.*

Figs. 1, 5, 8, 10–21 from a bed bug dissected forty-eight hours after feeding from the sore.
Figs. 2 and 6 from a second bug dissected twenty-four hours after feeding from the sore.
Figs. 3, 4, 7 and 9 from a third bug dissected forty-eight hours after feeding from the sore.

Fig. 1. Dividing Herpetomonas form. Size $11 \times 2{\cdot}5\ \mu$.
Fig. 2. Developmental form with flagellum not yet showing. Size $8{\cdot}5 \times 2{\cdot}5\ \mu$.
Fig. 3. Abnormal individual.
Fig. 4. Herpetomonas form showing connection of flagellum with cone-like prolongation of kinetonucleus. Size $10 \times 2\ \mu$.
Fig. 5. Abnormal division form with double nucleus. Size $14 \times 2{\cdot}5\ \mu$.
Fig. 6. Group of partially developed parasites. One has no nuclear apparatus.
Fig. 7. Two oval flagellate forms. Length $6{\cdot}5\ \mu$.
Fig. 8. Small abnormally dividing form. Length $4{\cdot}5\ \mu$.
Fig. 9. Curious dividing form without nucleus. Length $12\ \mu$.
Fig. 10. Evidently degenerating form.
Fig. 11. Flagellate with dividing nucleus. Size $14 \times 2\ \mu$.
Fig. 12. Large clump of developing parasites.
Fig. 13. Flagellate form. Size $12 \times 2\ \mu$.
Fig. 14. Abnormal or degenerating form. Size $16 \times 2{\cdot}5\ \mu$.
Fig. 15. Flagellate with large quantity of chromatin staining material in protoplasm Size $12 \times 2\ \mu$.

Fig. 16. Elongated form without evident flagellum. Karyosome shows within the nucleus. Length 9·5 μ.

Fig. 17. Typical flagellate form. Size $7 \times 2\ \mu$.

Fig. 18. Another and larger typical flagellate form. Size $14 \times 1\ \mu$.

Fig. 19. Small curved forms. Size $5 \times 2\ \mu$.

Fig. 20. Dividing forms without flagella. Size $5 \times 2\ \mu$.

Fig. 21. Dividing flagellate form. Karyosome in division. Size $10 \times 2{\cdot}8\ \mu$.

The sore parasite (Leishmania tropica).

Figs. 22–29 *from dried smears made direct from the sore and stained by Giemsa's stain.*

Fig. 22. Torpedo-shaped parasite with the kinetonucleus closely applied to the nucleus. Such a position of the two nuclei may lead to the erroneous idea that a fusion of these has taken place. The rhizoplast running towards the blunt extremity of the body is clearly shown.

Figs. 23 and 24. Two similar parasites with the kinetonucleus not so closely applied to the nucleus. These torpedo-shaped parasites are mostly extracellular.

Fig. 25. Typical parasite from the sore. The two parts of the kinetonucleus show well, one deeply and the other lightly staining. The rhizoplast arises from the pale staining part. Length 2·5 μ.

Fig. 26. Another and larger parasite in division. The pale half of the kinetonucleus shows up clearly. The new rhizoplast is already formed though it is still smaller than the original one. Length 5 μ.

Note. These parasites (Figs. 25 and 26) which are from dried films stained by Giemsa's stain, should be compared with the similar parasites stained by Heidenhain's iron haematoxylin method in films fixed in Schaudinn's fixative without any drying. Such forms are shown at Pl. XIII, figs. 9 and 15.

Figs. 27 and 28. Two abnormal forms without nuclei. Many of these are to be found in the smears from the sore.

Fig. 29. Form with dividing nucleus.

Figs. 30–36. *From cultures of the sore parasite on rabbit's blood agar.*

Fig. 30. Cultural form showing dividing karyosome in nucleus and dividing kinetonucleus connected with which is the pale staining dome-shaped structure from the apex of which the flagellum springs. Length 11 μ.

Fig. 31. Similar form with the dome-shaped structure on the kinetonucleus. A second rhizoplast is forming. Length 10 μ.

Figs. 32 and 33. Two parasites in division. They show well the kinetonucleus with its dome-shaped structure which divides with the kinetonucleus. The second rhizoplast is a new formation. Size $8 \times 5\ \mu$.

Fig. 34. Division form showing the dividing karyosome and kinetonucleus with its dome-shaped structure and newly-formed rhizoplast. Length 7 μ.

Fig. 35. Typical cultural form. The connection of flagellum to kinetonucleus is well shown. Length 12 μ.

Fig. 36. Form showing the kinetonucleus with a deeply staining dividing structure within it.

Figs. 37–41. *Developmental forms of the sore parasites in* Stegomyia fasciata.

Fig. 37. From a *Stegomyia fasciata* which had fed from the sore on ten successive days and was dissected forty-eight hours after the last feed.

Figs. 38, 39, 41. From a *Stegomyia fasciata* which had fed from the sore on four successive days and was dissected twenty-four hours after the last feed.

Fig. 40. From a *Stegomyia fasciata* which had fed from the sore on two successive days and was dissected twenty-four hours after the last feed.
Fig. 37. Form with two rhizoplasts. Length 5 μ.
Fig. 38. Form without flagellum. Length 6 μ.
Figs. 39 and 40. Partially developed forms. Length 4 and 5 μ respectively.
Fig. 41. Typical flagellate form. Length 7 μ.

PLATE XIII.

All the figures from preparations of the rabbit's blood agar cultures of the sore parasite fixed in Schaudinn's fixative and stained by Heidenhain's iron haematoxylin method, except figs. 9 and 15 which are from preparations made in the same manner direct from the sore.

Fig. 1. Typical cultural form. Length 13 μ.
Fig. 2. Dividing form very much discoloured. The fine line connecting the separating halves of the nuclear karyosome still retains the stain. Length 6 μ.
Fig. 3. Typical form showing a clear interval between the end of the rhizoplast and the kinetonucleus. Length 9 μ.
Fig. 4. Dividing form showing a condition of the kinetonucleus comparable with that of fig. 36, Pl. I. Length 7 μ.
Fig. 5. Dividing form showing two structures in the position of the kinetonucleus. Length 7 μ.
Fig. 6. Form in which the nucleus contains two chromatin masses, possibly the divided karyosome. Length 7 μ.
Fig. 7. Dividing form with two flagella. Length 8 μ.
Fig. 8. Dividing form showing the fine line connecting the halves of the karyosome. The kinetonucleus also dividing. The protoplasm shows commencement of division in the form of a groove between the rhizoplasts. Length 7 μ.
Fig. 9. Typical parasite from the sore. Compare with figs. 25 and 26 in Pl. XII, which have been dried and stained by Giemsa's stain.
Fig. 10. Dividing culture form. The halves of the kinetonucleus connected by the fine line like that which occurs in division of the karyosome of the nucleus.
Fig. 11. Form showing the flagellum connected with a dome-shaped structure so frequently seen in the dried films (cf. Pl. XII, figs. 30–35).
Fig. 12. Form with nuclear karyosome in division.
Figs. 13 and 14. Forms with dividing kinetonucleus.
Fig. 15. Parasite from the sore. Compare with fig. 9 and Pl. XII, figs. 25 and 26.
Fig. 16. Dividing form with division of kinetonucleus completed. The halves of the karyosome connected by the long drawn out line. Length 6·5 μ.

PLATE XIV.

Figs. 1 and 2. Typical appearance of the oriental sore as it occurs on the faces of children in Bagdad.
Fig. 3. Girl age 14 with oriental sore on the face and another on the right wrist. The sore on the face is of the type of a spreading ulcer ("female sore"), that on the wrist is a non-ulcerating growth ("male sore").
Fig. 4. The same case as fig. 3, showing the spreading ulcer on the face and its tendency to heal in one part as it spreads in another.

PLATE XV.

Lankesteria culicis (*Ross* 1898) *a gregarine of* Stegomyia fasciata.

Fig. 1. Two gregarines encysted in the malpighian tubes of the pupa.

Fig. 2. Nucleus of one of the gregarines of fig. 1, showing a process budding from the karyosome.

Fig. 3. Another gregarine cyst in the malpighian tubes of a pupa. The deeply staining body between the gregarines is the remains of the fixation organs which are applied to one another when association takes place.

Fig. 4. Section through a gregarine cyst. The nucleus of one of the gregarines shown is breaking up. In the next section in the series (fig. 6) is seen the beginnings of the first nuclear spindle.

Fig. 5. A gregarine in the gut epithelium of a larva. Length of gregarine 45 μ.

Fig. 6. See fig. 4.

Fig. 7. A later section from the same cyst as fig 1. The dark body arising from the fixation organs is shown well.

Fig. 8. A gregarine in the epithelium of the gut of a larva. Drawn on a larger scale than fig. 5. Length of gregarine 50 μ.

Fig. 9. Longitudinal section through a malpighian tube of a pupa showing many gregarine cysts. Note the excavations of the tube cells.

Fig. 10. Gregarine cyst in malpighian tube of pupa showing zygotes and residual protoplasm containing unused nuclei.

Figs. 11, 13, 15 and 16. Stages in the conjugation of the gametes and nuclear fusion. The unequal size of the nuclei of the conjugating gametes is shown well in figs. 11, 13 and 15.

Figs. 12 and 14. Gametes showing the clear hyaline pointed structure near the nucleus.

Fig. 17. Gregarine cyst from a malpighian tube of a pupa showing gametes.

Fig. 18. Formation of gametes by a process of budding.

Fig. 19. Gregarine cyst from a malpighian tube of a pupa. Last stage in nuclear multiplication. In one gregarine, very much ramified, the nuclei are ranged on the surface for gamete formation. In the other gregarine can be seen nuclear division spindles presumably of the last division. Many large unused nuclei are seen.

Fig. 20. Gregarine cyst from a malpighian tube of a pupa. The zygotes have become elongated preparatory to formation of sporocysts.

Figs. 21–27. Stages in the development of the sporocysts.

Figs. 28 and 29. Nuclear spindles of the third nuclear division of a gregarine.

Fig. 30. Karyosome free in protoplasm of the same gregarine from which figs. 28 and 29 were drawn.

Figs. 31 and 32. Two adjacent sections through a gregarine cyst from a pupa. Fig. 31 shows the breaking up karyosome and the complete disappearance of the nuclear membrane of the gregarine nucleus. Fig. 32 a structure which is probably the forming first nuclear spindle.

Figs. 33 and 34. Dividing nuclei (third division) from the gregarine which was associated with the one from which figs. 28–30 were taken.

Fig. 35. Longitudinal sections through a malpighian tube of the imago of *Stegomyia fasciata*. It is filled with liberated sporocysts.

PLATE XVI.

Development of Haemogregarina canis *in the dog and in the tick* Rhipicephalus sanguineus.

Fig. 1. Large öocyst from the tick. The nucleus of each sporocyst has multiplied till each possesses about 16.

Fig. 2. Cyst from the spleen of a dog containing schizont with protoplasm filled with refractile spheres and six nuclei.

Fig. 3. Cyst from the spleen of a dog containing three large merozoites and residual body.

Fig. 4. Young schizont in mononuclear cell in spleen. The nucleus of the parasite discoloured to show the karyosome.

Fig. 5. Schizont from spleen of dog. The nuclei show the karyosomes clearly.

Fig. 6. Cyst from spleen of dog similar to that at fig. 3 but more discoloured. The nuclei of the merozoites consist of a nuclear membrane over which fine granules are scattered. There is a central karyosome.

Fig. 7. Sporocyst from an öocyst in the tick. It shows the budding off of the sporozoites. Eight are formed at each pole. The drawing only shows four of these.

Fig. 8. Cyst from spleen of dog. The small forms are destined to enter the leucocytes and to appear in the peripheral circulation.

Fig. 9. First nuclear division of schizont in spleen of dog. The karyosome divides and functions as an intranuclear division centre.

Fig. 10. Sporocyst from an öocyst in the tick. It shows the eight nuclei arranged at one pole. Eight nuclei were similarly arranged at the other pole.

Fig. 11. Developing form in the tick. Possibly an early öocyst.

Fig. 12. A sporozoite from a sporocyst in the tick. Length 14 μ.

Fig. 13. Early schizont in spleen of dog. The nucleus shows the karyosome.

Fig. 14. Schizont from spleen of dog. Each nucleus has a karyosome.

Fig. 15. A form within an epithelium cell of the gut of the tick. Possibly a zygote.

Fig. 16. Large öocyst filled with sporocysts containing sporozoites from body of tick.

Fig. 17. Developing sporoblasts in öocyst from the tick.

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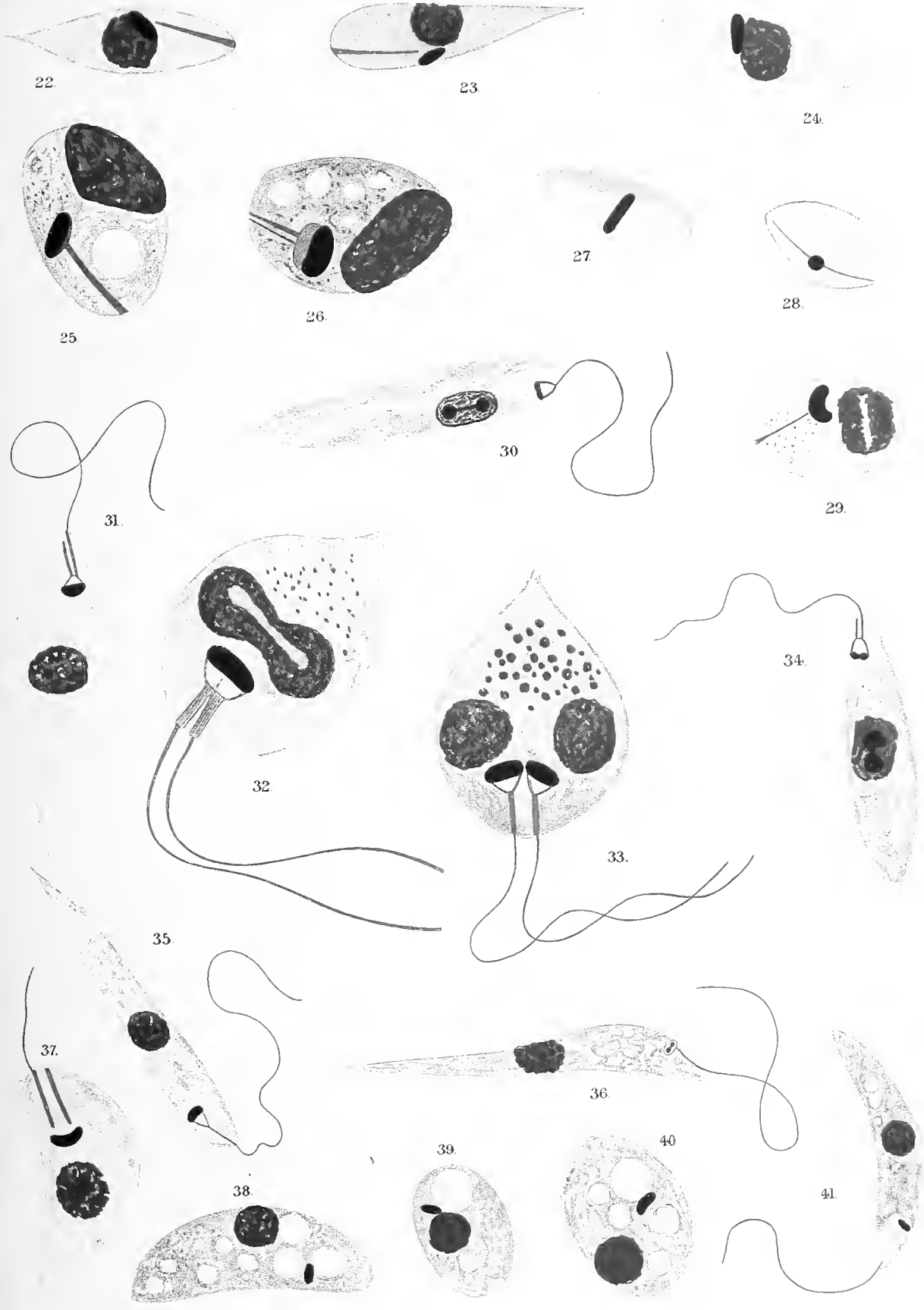
PLATE XII.



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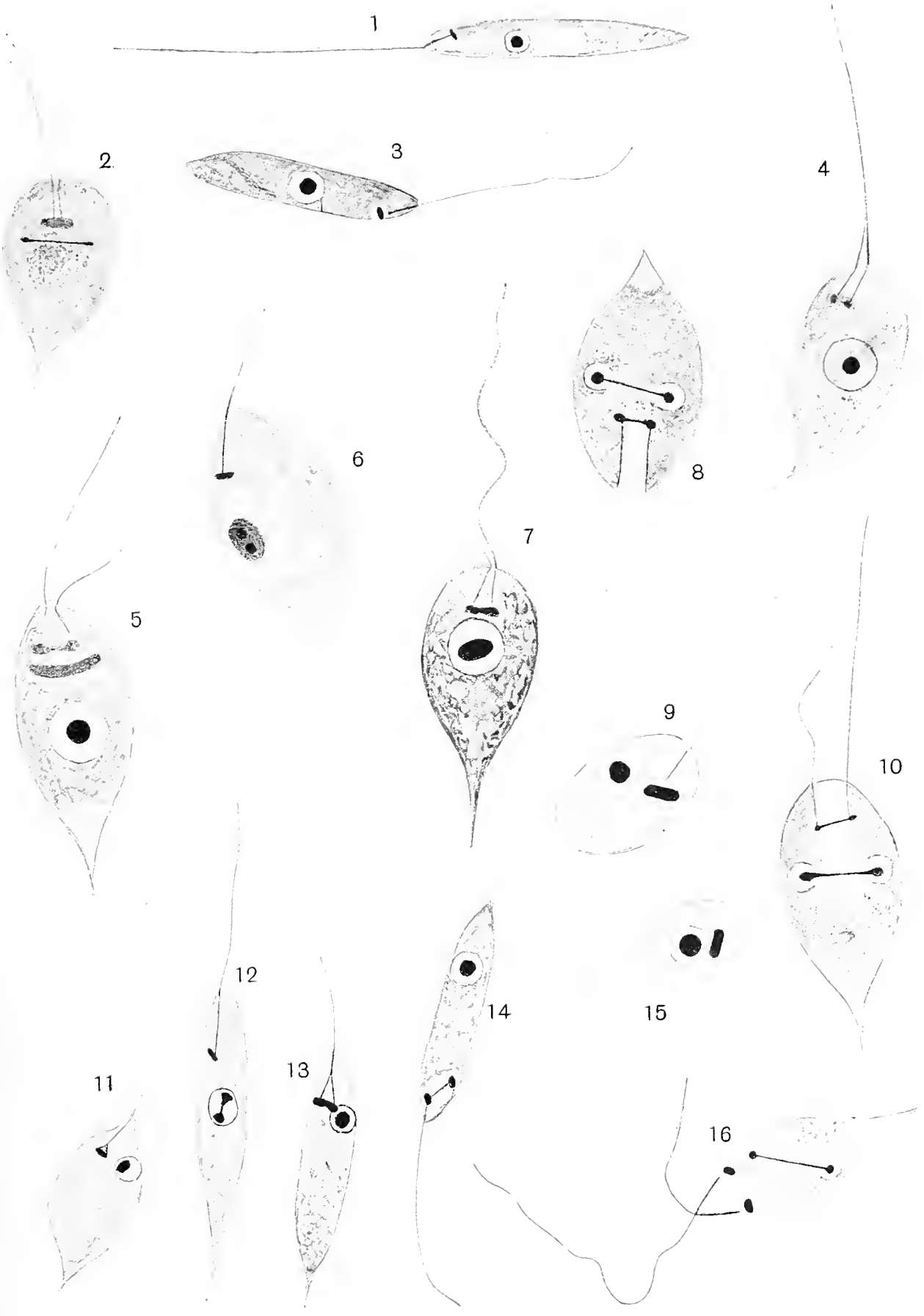

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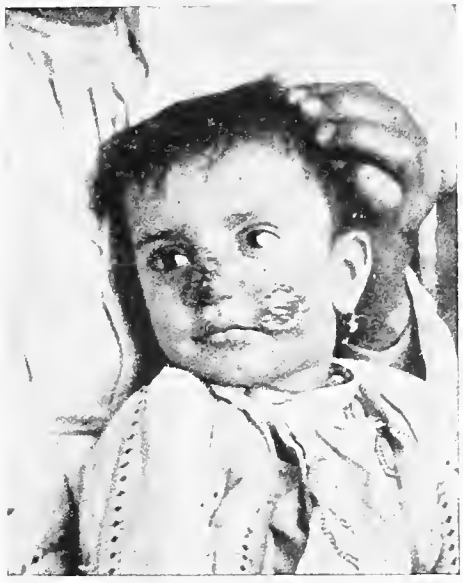

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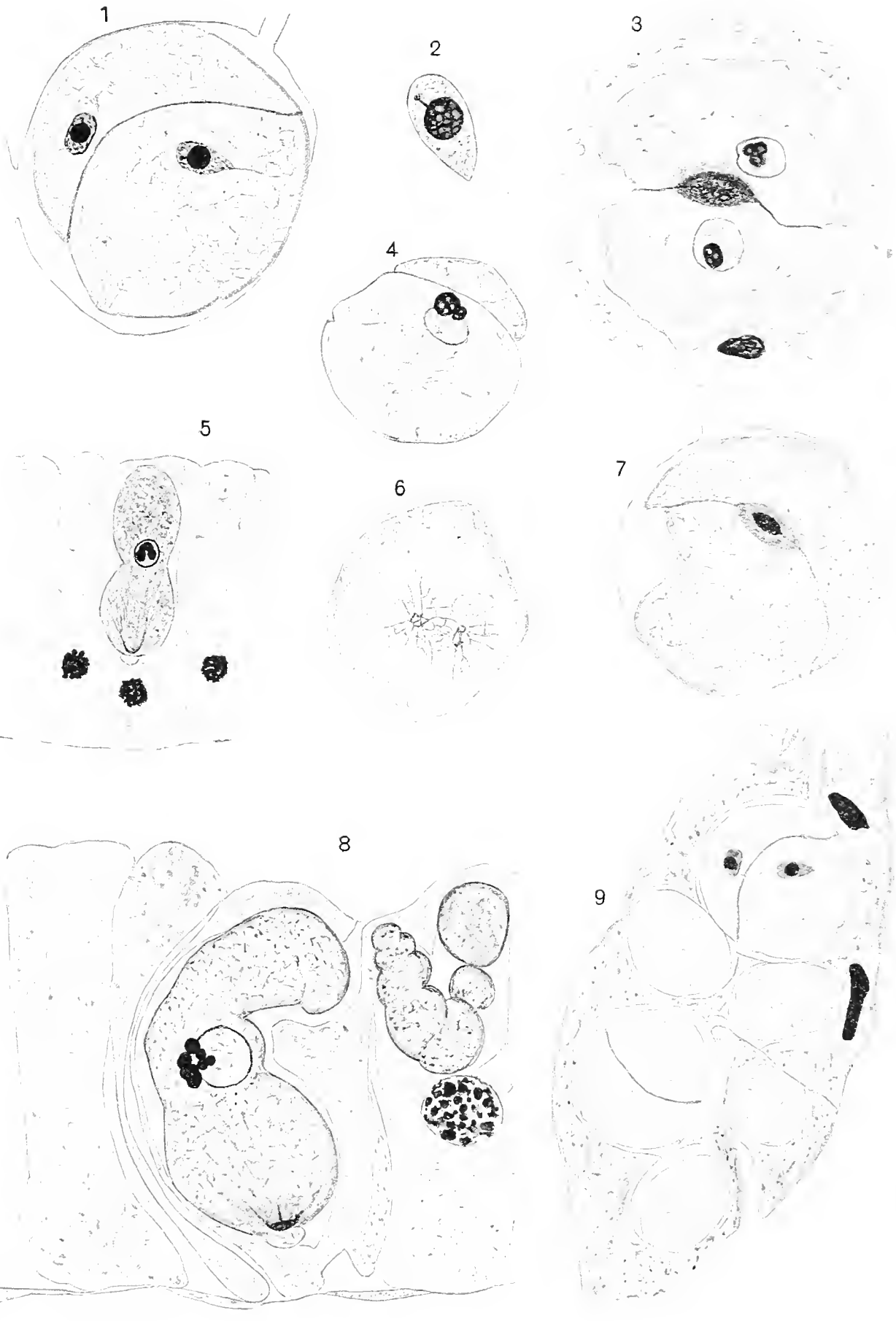

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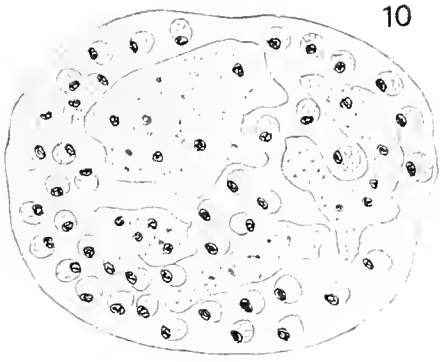
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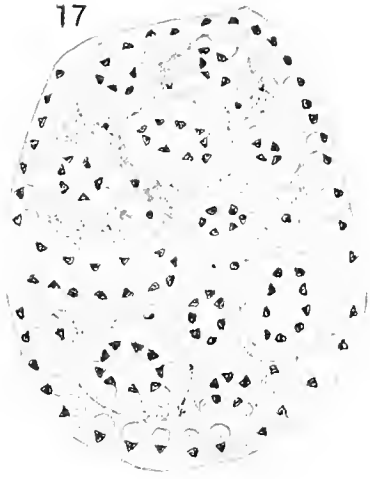
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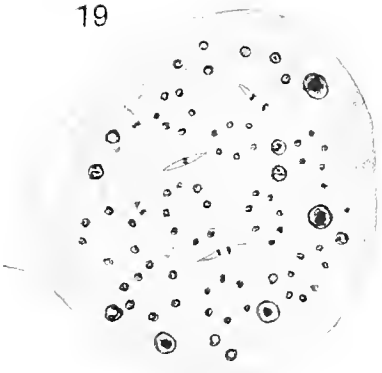
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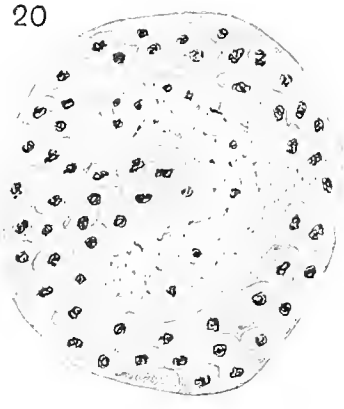
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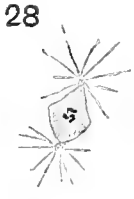
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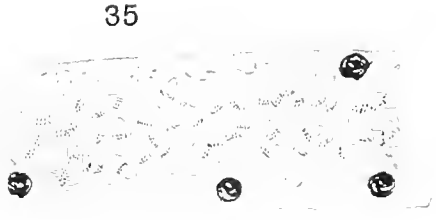
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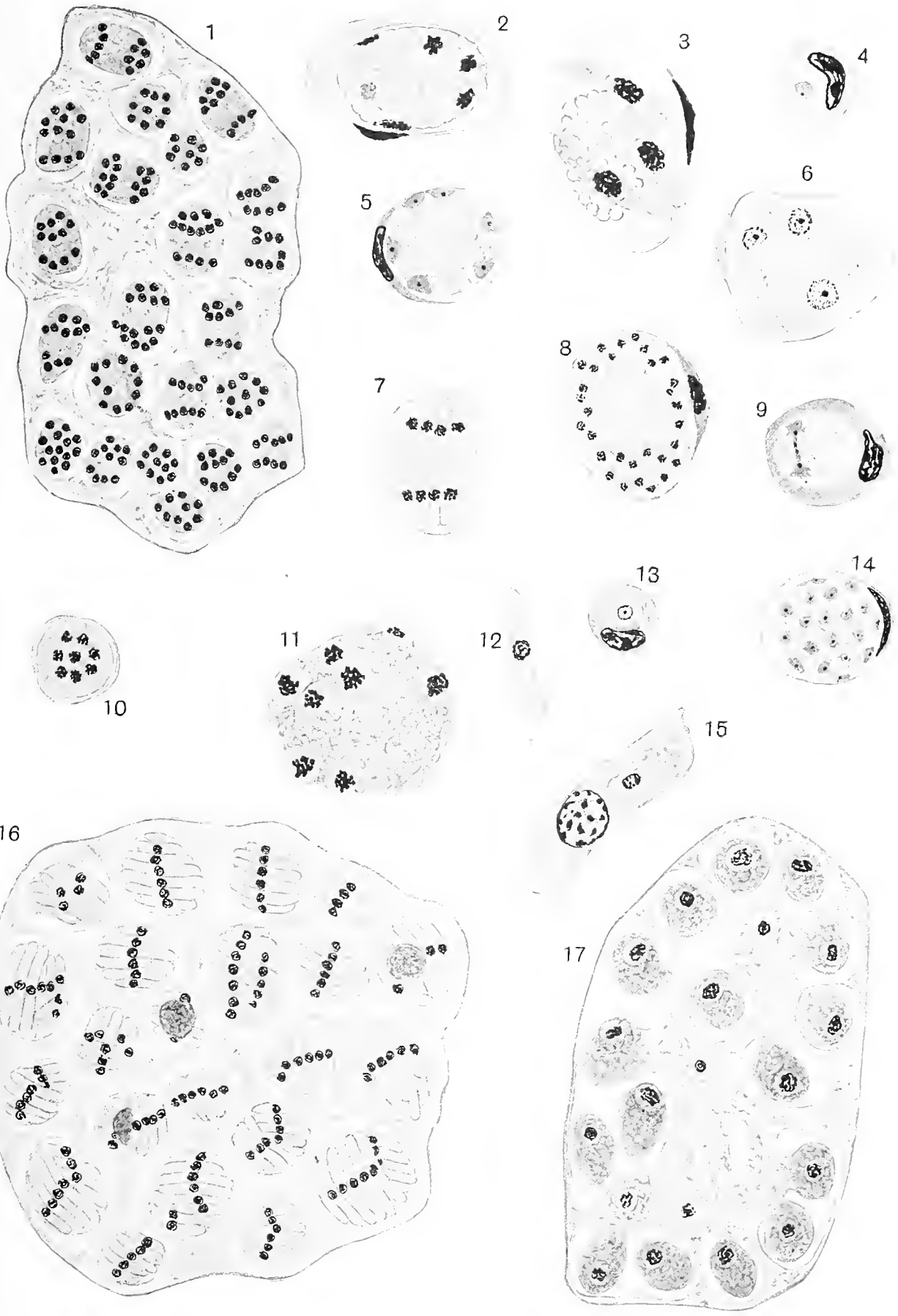

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